ACUTE MYELOID LEUKAEMIA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

Didintle Mokgoko
Student number: 0401000R

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

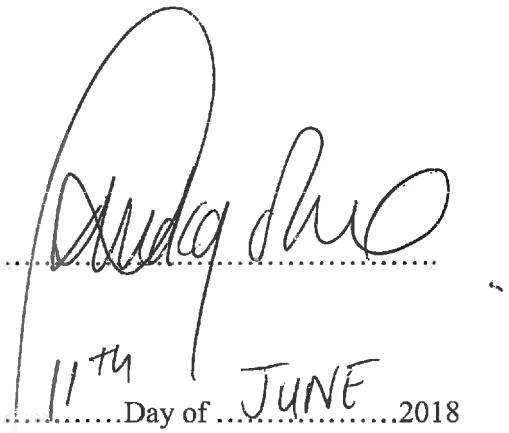
Johannesburg, 2018

ETHICS COMMITTEE APPROVAL

This study was approved by the Human Research Ethics Committee (Medical), of the University of the Witwatersrand. Clearance certificate number: **M141169**.

DECLARATION

I, DIDINTLE MOKGOKO, declare that this research report is my own work, which is being submitted for the degree Master of Medicine (in the dissertation format) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other University.



.....

11TH JUNE

.....Day of 2018

DEDICATION

Thank you to all my mentors, friends and family for all your support during this research.

To my mother and sister, thank you for your words of encouragement and for your ongoing faith in my success; present and future.

ABSTRACT

Background

Acute myeloid leukaemia (AML) is a haematological malignancy that results from the malignant clonal proliferation of myeloid progenitor cells. The clinical presentation of AML is a consequence of bone marrow infiltration and replacement of the normal cellular elements in the bone marrow, resulting in a reduced production of mature blood cells. In the era of antiretroviral therapy, with the resultant increase in longevity of HIV seropositive patients, it is becoming more important for clinicians to be aware of the increasing incidence of solid organ and haematological malignancies in this group of patients. To date, there are no local studies evaluating the rare entity of AML occurring in patients who are infected with HIV in South Africa.

Aims and Objectives

- To describe the demographics, clinical presentation, laboratory features and management of patients with AML, including HIV seropositive AML from 01/01/2005 to 31/12/2014
- To describe the demographics, clinical presentation, laboratory features and management of patients with HIV seropositive AML from 01/01/1993 to 31/12/2004, in the era of combined antiretroviral therapy not being available

Patients and Methods

A retrospective study in which patient records of adults with AML diagnosed in the Clinical Haematology Unit, Department of Medicine, at Chris Hani Baragwanath Academic hospital during the period 01/01/1993 to 31/12/2014 were reviewed. The data of patient's demographics, clinical presentation, laboratory results and management was collected and evaluated.

Results

A total of 195 patients with AML were evaluated. This included 33 HIV seropositive patients with AML. However, a direct comparison was only made with 27 HIV seropositive patients compared to seronegative patients during the period 01/01/2005

to 31/12/2014. The majority of patients were of Black African ethnicity (91.5%). There was a male predominance of 52% in the overall population, while the HIV seropositive AML subgroup showed a female predominance of 59%. The median age at presentation was 45 years (range 18-88 years). The clinical presentation was mainly with features of bone marrow failure/infiltration, manifesting with anaemia, infection and bleeding. The incidence of tuberculosis was significantly higher among the HIV seropositive AML patients. Extramedullary disease was found in 21% of patients with 7% of patients having a myeloid sarcoma. Although HIV seropositive patients with AML had lower white cell counts, haemoglobin and platelet counts compared to their HIV seronegative counterparts, this difference was not statistically significant. The most common histological subtypes across the study were AML (M2) in 25% and AML (M3) in 22% of the patients. The most common favourable cytogenetic abnormalities were t(15; 17) and t(8; 21), while the most common unfavourable cytogenetic abnormality was t(9; 22). For induction chemotherapy, patients were treated with the standard "3+7" regimen, which consists of a combination of an anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 7 days. Complete remission was achieved in 60% of all patients who received induction chemotherapy. The most common consolidation therapy given was the combination of an anthracycline (daunorubicin) and high dose cytosine arabinoside. Approximately 18% of the patients were given palliative chemotherapy. The overall patient outcomes were as follows: 76% of the patients demised, 18% of the patients were lost to follow up, and 6% were alive.

Conclusion

Acute myeloid leukaemia is the most common acute leukaemia seen in adults. AML in association with HIV is uncommon. To our knowledge, the current study encompasses the largest single center experience of this association. HIV seropositive patients with AML present at a younger age, with a slight female predominance. In general, the clinical presentation, treatment and outcome are similar to HIV seronegative AML, with a few exceptions. The similarities and differences are highlighted in this research report. It is hoped that the findings of this retrospective study will form the basis for more detailed and focused prospective studies on the association of AML in HIV seropositive individuals.

ACKNOWLEDGEMENTS

My most heartfelt thank you to my impeccable supervisors, Professor Moosa Patel and Dr Atul Lakha. Thank you for your patient tutelage and guidance throughout this process. I could never fully convey my gratitude for your selfless dedication and time spent mentoring me on this journey. Thank you for continuing to inspire me to not only be a good clinician but an even better human being.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OFABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AML	Acute Myeloid Leukaemia
ARV	Antiretroviral
cART	Combination antiretroviral therapy
CEBPAα	CCAAT/Enhancer Binding Protein alpha
CCR5	Cysteine-cysteine receptor 5
CD_4	Cluster of differentiation 4
CHBAH	Chris Hani Baragwanath Academic Hospital
CLAG	Cladrabine Ara C GCSF
CML	Chronic Myeloid Leukaemia
CNS	Central nervous system
DIC	Disseminated intravascular coagulopathy
FAB	French American British
FBC	Full Blood Count
FLAG	Fludarabine Ara C GCSF
FLT3	Fms related tyrosine kinase 3
GCSF	Granulocyte Colony Stimulating Factor
GIT	Gastrointestinal tract
GUT	Genitourinary tract
HAART	Highly active antiretroviral therapy
HB	Haemoglobin
HCT	Haematocrit
HIV	Human immunodeficiency virus
HIVVL	HIV viral load
HLA	Human leucocyte antigen
Inv (16)	Inversion 16
LDH	Lactate dehydrogenase
MCV	Mean cell volume
MRD	Minimal residual disease
MSK	Musculoskeletal
NA	Sodium
NPM	Nucleophosmin

PLT	Platelet
PO4	Phosphate
PTB	Pulmonary tuberculosis
RESP	Respiratory
SA	South Africa
SCT	Stem cell transplant
SSA	Sub-Saharan Africa
t(8; 21)	Translocation chromosome 8 to 21 (RUNX1-RUNX1T1 gene)
t(9: 22)	Translocation chromosome 9 to 22 (BCR-ABL gene)
t(15; 17)	Translocation chromosome 15 to 17 (PML-RARA gene)
TB	Tuberculosis
TB ABDO	Tuberculosis of the abdomen
TBM	Tuberculous meningitis
WCC	White cell count
WHO	World Health Organization

CHAPTER 1: LITERATURE REVIEW

1.1. Haematopoieisis and Acute Myeloid Leukaemia

In the human, haematopoieisis is the process by which haematopoietic stem cells within the bone marrow commit to a particular cellular lineage and differentiate to form the mature cellular components of blood. Haematopoetic stem cells differentiate into one of two major cellular lines, the myeloid or lymphoid lineages. The lymphoid lineage will result in the formation of mature T and B lymphocytes as well as plasma cells that are essential to the functioning of cellular mediated and humoral immunity. The myeloid lineage gives rise to mature erythrocytes, granulocytes and platelets. Granulocytes, which consist of neutrophils, eosinophils and basophils as well as monocytes, form the innate immune system. This process of cellular differentiation and maturation is both stimulated and regulated at a molecular level by colony stimulating growth factors. Cytogenetic abnormalities that occur at the level of the haematopoietic precursor cell can alter normal cellular proliferation and differentiation, resulting in an accumulation of immature myeloid or lymphoid precursors within the bone marrow and peripheral blood (1). The clonal proliferation and marrow infiltration by these abnormal precursor cells, with the inevitable displacement of normal haematopoetic elements, results in leukaemia.

1.2. Pathogenesis of Acute Myeloid Leukaemia

Acute Myeloid Leukaemia (AML) is a result of malignant clonal proliferation of myeloid progenitor cells. Any genetic abnormalities that occur during and affect the growth and transcription factors of these cells, renders them incapable of differentiating into mature cells (1). These immature myeloid precursors (blasts) accumulate in the bone marrow and blood resulting in the reduced production of mature erythrocytes, neutrophils, monocytes, eosinophils, basophils and platelets. The clinical presentation of Acute Myeloid Leukaemia is a consequence of bone marrow (medullary) and tissue (extramedullary) infiltration by these blasts.

1.3. Acute Myeloid Leukaemia risk factors

The development of AML may be a primary (de novo) or a secondary process. Some well documented risk factors for the development of secondary AML include: previous exposure to benzene, transformation of a preceding myelodysplastic process or myeloproliferative neoplasm, as well as exposure to ionizing radiation (1, 2). As many as 10-15% of patients develop AML following exposure to a chemotherapeutic agent, for a primary solid organ malignancy including lymphoma. The most common drugs that are implicated in therapy related AML are alkylating agents, doxorubicin and etoposide (1). Leukaemic cells in secondary AML have accumulated multiple cytogenetic and molecular abnormalities that tend to confer resistance to conventional chemotherapy regimens; these patients are therefore more likely to have adverse outcomes with a higher risk of relapse or refractory disease (1, 3).

1.4. Epidemiology

Acute Myeloid Leukaemia (AML) is the most common type of acute leukaemia in the adult population (4). In the Western world, AML is typically a disease of middle aged and elderly individuals with a median age at presentation of 67 years (5). In the United States and Europe, the incidence has been stable at 3 to 8 cases per 100,000 population per year (1). Historically, the disease has a higher incidence among male compared to female patients, with a male: female ratio of approximately 3:2 (1). There is currently limited population based data with regards to the epidemiology of Acute Myeloid Leukaemia in Africa and particularly South Africa. However, in a recent study of a South African cohort of 160 cases of de novo AML by Marshall et al., it was found that there was a slight female predominance of 52% and a younger median age at diagnosis of 41 years (6).

1.5. Classification of Acute Myeloid Leukaemia

There are two main classification systems for Acute Myeloid Leukaemia. The most widely used is the French American British (FAB) classification. The FAB classification considers both the morphology and the cytogenetic abnormalities of the malignant haematopoietic cell and divides patients into subclasses, namely M0-M7.

The World Health Organization (WHO) classification considers the immunological, morphological and cytogenetic characteristics of the disease and further specifies AML that has evolved from pre-existing myelodysplasia, myeloproliferative neoplasms or therapy related AML (7, 8). New subclasses are constantly being defined as ongoing research discovers new genetic and molecular aberrations and the most recent updated WHO classification of myeloid neoplasms and acute leukaemias was published in 2016 (8).

1.6. Acute Myeloid Leukaemia presentation

The clinical presentation of patients with AML is primarily with features of bone marrow infiltration and bone marrow failure due to the inability of the malignant blasts to differentiate into mature erythrocytes, granulocytes and platelets (1). Symptoms and signs include varying degrees of anaemia, bleeding and susceptibility to infections. Clonal blast proliferation with rapid expansion of marrow spaces may result in bone pain. Less commonly, malignant myeloid blasts infiltrate extramedullary tissues and patients may present with gum hypertrophy, organomegaly and skin manifestations, such as granulocytic/myeloid sarcomas. Myeloid sarcomas, also known as granulocytic sarcoma or chloroma, are soft tissue masses that are formed by aggregation of myeloid blasts in peripheral tissues. Myeloid sarcomas are generally rare and represent less than 1-5 % of the clinical presentation of AML (1). However, they may be more commonly associated with particular histological subtypes of AML, such as AML M2 (1).

1.7. Complications of Acute Myeloid Leukaemia

Acute Myeloid Leukaemia can present with complications at the time of diagnosis. A high blast load and infiltration of peripheral tissues can impair blood flow, resulting in poor tissue perfusion. This complication of leukaemia is known as leukostasis and is more likely to occur in patients with extremely elevated white cell counts. Leukostasis may be life threatening when it occurs in the lungs, resulting in acute respiratory distress syndrome, and in the central nervous system. Bleeding and coagulopathy may be a complication of disseminated intravascular coagulopathy (DIC). This

complication is more commonly associated with the acute promyelocytic subtype of AML, AML M3 (1). The initiation of remission inducing cytotoxic therapy may be associated with further worsening of cytopenias and tumour lysis syndrome, particularly in patients presenting with markedly elevated leucocyte counts (generally above 200 x 10^9/l).

1.8. Diagnosis of Acute Myeloid Leukaemia

Although a presumptive diagnosis of AML can often be made based on the clinical features of bone marrow failure and the presence of myeloid blasts on a peripheral blood specimen, the definitive diagnosis of AML is confirmed on the morphological assessment of a bone marrow aspirate and trephine sample. The blasts should be confirmed to be of myeloid origin and must account for at least 20% of the total cell population within the bone marrow (7, 8). In a recent review of the diagnostic criteria for AML it has been stated that a diagnosis of AML can be made at any blast count in the marrow, provided that typical cytogenetic abnormalities are demonstrated within the malignant cells, including t(15; 17), t(8; 21), t(16; 16) and inv(16) (2, 7, 8). Further investigation of immunophenotying by means of flow cytometry, in addition to molecular and cytogenetic testing can then be carried out to determine specific genetic abnormalities. This aids in further classifying the subtype of AML. Determining the morphological and cytogenetic subtype of AML aids in the diagnosis, treatment (choice of chemotherapy) and the post treatment monitoring of patients with AML (2).

The presence of particular cytogenetic abnormalities are directly associated with either a good, intermediate or poor prognosis for the response to chemotherapy as well as the overall disease outcome (1, 9, 10). The cytogenetic abnormalities that confer the best prognosis and response to chemotherapy are those consisting of balanced fusion genes such as t(8; 21), t(15; 17) and inv(16) (2). Unfavourable cytogenetic abnormalities are associated with high risk for chemotherapy resistance, and include: t(9; 22), t(6; 9) and a complex karyotype (i.e. 3 or more chromosomal abnormalities occurring within one cell). These patients have lower remission rates and higher risk of disease relapse. Patients with genetic abnormalities that are

associated with intermediate risk disease show a variable response to chemotherapy and the prognosis in these patients may be somewhat unpredictable. Novel cytogenetic abnormalities such as genetic mutations in nucleophosmin-1 (NPM1), fms related tyrosine kinase 3 (FLT3) as well as ccaat/enhancer binding protein α (CEBPα) have been identified in patients with AML and are being used to aid in further subtyping and risk stratifying patients with AML (11).

1.9. Management of Acute Myeloid Leukaemia

1.9.1. Supportive measures

The supportive management of AML consists of the following:

I. Psychosocial and educational support of the patients and their family,

II. The transfusion of blood and blood products as and when indicated. Blood products should be leucocyte depleted to prevent alloimunisation in patients who may be potential candidates for stem cell transplantation, once remission has been achieved (4).

III. The management of neutropenia and infections. Severe neutropenia manifests as an increased susceptibility to infections and remains an important complication and cause of mortality in patients undergoing chemotherapy for AML (2). Neutropenic patients are at risk of potentially invasive and disseminated infections by gram-positive and gram-negative bacteria, as well as fungal and atypical pathogens (1). To minimize the risk of infection, neutropenic measures include isolation, barrier nursing and prophylactic antibiotic and antifungal therapy as required.

IV. Other general measures: these include treating and correcting electrolyte abnormalities, prevention of hyperuricaemia, pain management with analgesics and leukapharesis, where indicated. Patients who present with excessively elevated leucocyte counts with symptoms of leukostasis may require rapid lowering of the blast count with leukapharesis (12).

In the past granulocyte transfusions were explored as a potential solution to the challenge of severe neutropenia, however they proved to be technically difficult to procure, and their therapeutic benefit remains unclear (12). The routine use of granulocyte colony stimulating growth factors in myeloid neoplasms is not advisable due to the potential risk of stimulating further proliferation of the malignant myeloid

clone. However, they may occasionally become necessary to treat severe systemic infections during the induction phase of chemotherapy (4). The advent of improved antimicrobial agents and safer blood transfusion practices over time have greatly contributed to improving the survival and outcome of patients with AML, while the aim of initial definitive treatment is to rapidly reduce the malignant proliferation of blasts and restore normal haematopoiesis using a chemotherapeutic regimen (4).

1.9.2. Specific chemotherapy

Traditionally there are two phases of chemotherapy in the treatment of AML. The first phase is induction and is aimed at rapidly reducing the blast burden and inducing remission; the second phase is aimed at consolidating remission (once achieved), in order to maintain remission. Maintenance therapy is generally not administered to AML patients, with the exception of AML M3 (acute promyelocytic leukaemia).

The induction chemotherapy protocols for AML have remained fairly constant in the past decade, with the rates of complete remission ranging from 50-85% (4, 13, 14). A standard treatment regimen consists of the combination of an anthracycline, typically daunorubicin at 45-50 mg/m^2 given intravenously daily over 3 days with cytosine arabinoside at 100-200 mg/m^2 given as a continuous infusion over 7 days (4, 14). In suitable patients who have high-risk disease and are transplant eligible, allogeneic stem cell transplantation should be offered as a post remission strategy. In recent years, the need to achieve improved rates of complete remission and patient survival has prompted new regimens to be considered in the treatment of AML.

Dose intensification of daunorubicin up to 90 mg/m^2 has been shown to improve both the rates of complete remission as well as overall survival without significantly increasing the risk of toxicity in patients treated for AML (13). This benefit was initially mainly seen in younger patients (< 60 years of age) with a favourable or intermediate risk cytogenetic profile (13, 15). However, at long term follow up, the benefit of dose intensification of daunorubicin is seen in all age groups and across all cytogenetic profiles (13, 16-18). Locally, similar responses to dose intensification were found during two prospective studies by Novitzky et al., conducted at the University of Cape Town between 1990 and 1998, where higher doses of

daunorubicin (up to 75 mg/m^2) given during induction as well as consolidation cycles of chemotherapy, resulted in higher remission rates from 59% to 77% without adverse toxicity (19).

Alternative strategies giving higher cumulative doses of daunorubicin over a two-step induction programme have recently been studied by the United Kingdom National Cancer Research Council, but has been shown to have high toxicity and unacceptable 60-day mortality rates (13). The choice of anthracycline, in particular idarubicin compared to daunorubicin, has not shown any superiority in terms of remission rates and overall survival. In fact, idarubicin has been associated with potentially higher rates of marrow toxicity and longer hospital stays (4, 13).

New drugs that are targeting molecular abnormalities in the malignant stem cell have emerged recently in the treatment of AML. In particular, anti-CD 33 monoclonal antibodies targeting the transmembrane receptor have been used as additional therapy to the conventional induction chemotherapy regimen (20). Recent results of gemtuzumab-ozogamycin (Mylotarg®) from the UK Medical Research Council (MRC) 15 trial has only shown significant improved survival in patients with a favourable cytogenetic profile but has had no improvement in either remission rates or overall survival rates (13). Higher doses of the drug are also associated with life-threatening adverse effects such as veno-occlusive disease. This has prompted the need to either decrease the dose of the drug or to develop less toxic anti-CD 33 monoclonal antibodies (13).

There are a number of molecular targeted therapies that are at different stages of development and use in clinical trials, but are not yet generally available for use in standard clinical practice. For example, FLT3 tyrosine kinase inhibitors such as midostaurin, are being evaluated in combination with conventional chemotherapy and so far have shown some improvement in disease free but not overall survival rates (3). These novel therapies provide hope of improved remission rates and overall outcomes for patients with AML in the future.

In order to satisfy the definition of complete morphological remission, the following end points should be met: i) there should be no clinical evidence of disease, ii) there should be evidence of marrow recovery with an absolute neutrophil count more than 1 x 10^9/l, as well as a platelet count of more than 100 x 10^9/l, and the absence of blasts in the peripheral blood, iii) the bone marrow should consist of no more than 5% blasts (this should concur with flow cytometry showing $\leq$ 5% blasts). In addition, there should be evidence of tri-lineage maturation of haematopoiesis, and no evidence of foci or clusters of blasts on the bone marrow trephine biopsy (1). The cytogenetic abnormalities that were previously present should ideally not be detectable through cytogenetic and molecular testing, in order to define cytogenetic remission. Minimal residual disease (MRD) occurs in patients who meet the remission criteria but have evidence of persistent disease that is detectable by flow cytometry or genetic tests such as quantitative polymerase chain reaction (21). The presence of MRD suggests that there is a subset of clonal cells that are likely resistant to chemotherapy which have the potential to proliferate and cause future disease relapse (22). Patients with MRD have a higher risk of chemo-resistant and refractory disease and therefore have more adverse outcomes when compared to patients who have no evidence of molecular disease during complete remission (2, 21, 22). MRD is becoming an increasingly recognized prognostic factor in stratifying patients with AML in clinical practice (11, 22). Once remission has been achieved, post remission treatment ideally aims to eradicate the disease and in so doing cure the patient. However, this is a less realistic goal in elderly patients, who constitute a sizeable component of the AML population in the Western world.

The standard of care for consolidation chemotherapy in AML for patients with an intermediate to favourable risk profile is with cytosine arabinoside given at high doses of 1-3 g/m^2 twice a day (14). Data from the Cancer and Leukaemia Group B (CALB) suggests that at least 4 cycles of consolidation chemotherapy should be given for the best outcome (14). In patients with an unfavourable cytogenetic profile, an allogeneic haematopoetic stem cell transplant instead of consolidation chemotherapy should be considered as soon as complete remission has been achieved. This is due to the high risk of future relapse in this group of patients.

In patients who fail to respond to conventional induction chemotherapy or relapse during the course of their disease, salvage chemotherapy may be indicated although the choice of drug regimen remains a challenge. There is yet to be a combination of chemotherapy that has shown superiority as salvage therapy, however, treatment with a purine analogue (either a cladrabine or fludarabine) based induction regimen is the accepted conventional therapy in fit patients (2). These drugs are not effective as monotherapy and are combined with cytosine arabinoside and granulocyte colony stimulating factor (G-CSF), as the CLAG or FLAG regimen (23). In a recent study by Park et al., which evaluated patients with refractory or relapsed AML, treated with either cladrabine or fludarabine, there was no significant difference in overall and disease free survival between the two drugs (23). Alternatives for salvage therapy include various combinations of cytosine arabinoside with agents such as mitoxantrone, etoposide and idarubicin. In general, the outcome for patients with refractory or relapsed disease is poor with less than 10% of patients achieving remission with salvage therapy, and allogeneic stem cell transplantation being the only possibility of cure (2, 3, 23, 24).

As part of post remission therapy patients should ideally be considered for allogeneic stem cell transplantation (SCT) as this mode of definitive therapy results in the lowest risk for future disease relapses (13, 14, 25, 26). The limitation of allogeneic SCT, is the transplant related morbidity and mortality, related to myeloablation and potential acute or chronic graft versus host disease (26). In the South African setting, a major challenge is the paucity of HLA compatible donors for African patients (25). Patients who do not have an HLA compatible sibling or donor, can be considered for an autologous stem cell transplant if a matched unrelated donor transplant is not feasible.

The outcome of allogeneic compared to autologous stem cell transplantation as consolidation therapy in AML patients was assessed as part of the Cape Town Regimen V (CTR-V) prospective study. The data supports the fact that the outcomes and relapse rates of autologous SCT were comparable to those of allogeneic SCT in patients with favourable to intermediate risk cytogenetics (25). Where an allogeneic stem cell transplant is not possible, an autologous stem cell transplant maybe an alternative that can be used as consolidation therapy in these patients, albeit with the risk of disease relapse being higher in autologous transplants. Stem cell

transplantation remains a scarcely available mode of therapy in resource poor settings, as it requires highly specialized resources and can only be performed in experienced specialized centers.

1.10. Acute Myeloid Leukaemia in the elderly

Elderly patients (above the age of 60 years) with AML have a poor outcome with complete remission rates of less than 60% and survival rates at 5 years of 5-10% (3, 5, 27). There are multiple patient and disease related factors that contribute to the mortality of older patients with AML. Older patients with AML are likely to have severe co-morbidities and a poor performance status resulting in a decreased capacity to tolerate intensive chemotherapy protocols. The elderly patient with AML also presents a particular therapeutic challenge as they also have a higher risk of demonstrating unfavourable cytogenetics that are more likely to confer resistance to chemotherapy, prior exposure to radiation or chemotherapeutic agents during treatment of a previous malignancy, and secondary AML that evolved from pre-existing myelodysplastic disorders (27). This subset of patients are sometimes excluded from treatment with conventional chemotherapy, due to the risk of life-threatening therapy related toxicity and mortality. Elderly and very elderly (above the age of 75 years) patients are often treated with supportive measures, less intensive or palliative chemotherapy. Recent new therapies that have demonstrated varying benefits in younger patients with AML have not extended similar benefits to older patients, and the median survival remains at less than 12 months (5).

In AML patients who are unlikely to tolerate intensive chemotherapy, including the elderly, hypomethylating agents may be considered. These drugs include azacitidine and decitabine, have shown improved survival in preliminary trials when compared to conventional therapies such as low dose cytosine arabinoside, conventional intensive chemotherapy and supportive care in this group of patients (2, 3, 28).

1.11. Haematological malignancies in HIV seropositive adults

The relationship between haematological malignancies and HIV infection has been extensively documented in relation to lymphoid malignancies. HIV seropositive patients with advanced retroviral disease have a higher risk of developing lymphoproliferative disorders, particularly high grade B-cell Non Hodgkin Lymphoma (NHL), such as Diffuse Large B-cell, Burkitt Lymphoma and Plasmablastic Lymphoma which are all acquired immune deficiency syndrome (AIDS) defining malignancies (29-32). These are the most common haematological malignancies that are diagnosed in HIV seropositive patients in South Africa (33). In the past decade at Chris Hani Baragwanath hospital, the number of new HIV seropositive patients with NHL has increased from 25 to more than 100 patients per year. In addition, HIV seropositivity has increased to involve more than 70% of patients with NHL and over 50% of patients with Hodgkin Lymphoma (HL) (33, 34). HIV seropositive patients with lymphoproliferative disorders are younger than their HIV seronegative counterparts and they frequently present with advanced and widespread disease, often at unusual sites (34). The predominance of extranodal and bulky disease occurs more frequently in HIV seropositive patients (29, 34-36). These features constitute poor prognostic factors that contribute to adverse outcomes and poorer survival rates in patients who are HIV seropositive, when compared to those who are HIV seronegative (34).

With the introduction of highly active antiretroviral therapy (HAART) and combination antiretroviral therapy, the increase in the incidence of Non Hodgkin Lymphoma has stabilized. However, a steady increase in non-AIDS defining malignancies such as Hodgkin Lymphoma, has been observed in the HIV seropositive population (33, 37). The rise in non-AIDS defining malignancies is in part attributed to the improved survival of HIV seropositive patients on antiretroviral therapy. With regard to chronic leukaemias, it has been documented that HIV seropositive patients with Chronic Myeloid Leukaemia (CML) in the South African setting tend to have more atypical and aggressive disease (38). The association of CML with HIV infection is regarded as coincidental rather than causal.

In 2012, an estimated 35,3 million people were living with HIV globally (39). The highest burden of the HIV pandemic is in Sub-Saharan Africa. HIV seropositive patients presenting with various combinations of cytopenias is a common problem encountered in clinical practice in South Africa. In this setting the aetiology of the cytopenias are often multifactorial, including opportunistic infections, advanced retroviral disease, therapy related, nutritional factors, complicating malignancies and HIV associated myelodysplasia. Although myelodysplasia is a recognized risk factor for the development of AML, malignant transformation of HIV associated myelodysplasia to AML is a rare entity (36)

1.12. Acute Myeloid Leukaemia in HIV seropositive adults

1.12.1 Epidemiology

The epidemiology of AML in the setting of HIV infection is not well documented both internationally and locally. The international literature on this topic has focused on a mainly homosexual, male population, with a long-standing prior diagnosis of HIV infection, who develop AML later in life. The demographics of these patients differ from the South African burden of HIV, which classically occurs in heterosexuals, with a slight female predominance, and therefore limits extrapolation of the international data to the South African population.

1.12.2 Demographics

In the published data, the majority of HIV seropositive patients with AML are male. In a French study by Sutton et al., 13 out of 16 patients were male who had acquired HIV infection through homosexual contact (40). Aboulafia et al., described similar results during a multicenter retrospective review of 47 HIV infected adults diagnosed with AML. In this study, 39 out of 47 patients were male. The median age of presentation of 38 years for HIV seropositive patients with AML, was younger when compared to their HIV seronegative counterparts (36). AML (M2) and (M4) are the most common histological subtypes encountered in HIV seropositive patients with AML (36, 40, 41).

1.12.3 Aetiology

Although some patients with HIV may develop AML as a secondary process, the majority of HIV infected patients develop AML as a primary or de novo process with no identifiable risk factors. A significant risk factor for secondary AML that has been identified among these patients includes exposure to previous chemotherapeutic agents, particularly etoposide. This exposure often occurs during the treatment of a preceding AIDS-defining malignancy such as Kaposi's sarcoma or High grade B-cell Non Hodgkin Lymphoma (36). HIV infection and its interaction with bone marrow elements may result in myelodysplasia, often presenting clinically as varying degrees of cytopenias. However, the leukaemic transformation of HIV associated myelodysplasia to AML remains uncommon (36). It has also been suggested that in the setting of impaired immune surveillance within the marrow of HIV seropositive patients, leukaemic cells are not detected and cleared as efficiently as they would in an immune-competent patient, posing as a risk factor for the potential development of leukaemia.

1.12.4 Clinical Presentation

The clinical presentation of AML with features of bone marrow failure/infiltration is universal, regardless of the HIV status. However, the diagnosis of AML may be delayed in patients who are HIV seropositive due to the frequent occurrence of cytopenias among these patients, and that may be explained by alternative causes (36). The presentation of HIV associated malignancies (particularly Lymphoma), has been well documented to follow an atypical pattern, often presenting with disease that is more aggressive, more advanced and manifesting at unusual sites (34). Extrapolating from the HIV-lymphoma data, it may be expected that HIV seropositive patients with AML would be more likely to have extramedullary disease. However, this has not been conclusively demonstrated (36, 40).

1.12.5 Therapy and Outcomes

The natural history of HIV infection results in patients acquiring significant immune deficiency, putting them at risk for a myriad of opportunistic infections. Additionally, some patients have a poor performance status and/or varying degrees of cytopenias. These factors provide a particular challenge with regard to the tolerance of intensive chemotherapy regimens in the treatment of patients who are HIV seropositive with

AML. HIV seropositive patients with early stage HIV disease, who are well enough to receive conventional induction chemotherapy with the combination of an anthracycline and cytosine arabinoside ("3+7" regimen – 3 days of an anthracycline and 7 days of cytosine arabinoside), achieve similar complete remission rates as their HIV seronegative counterparts (36, 40). During consolidation therapy, HIV seropositive patients may require dose adjustment and shortened courses of consolidation therapy due to a higher incidence of prolonged neutropenia after the administration of induction chemotherapy (40). HIV associated opportunistic infections, particularly tuberculosis and pneumocystis jiroveci pneumonia, may occur during the course of chemotherapy. Therefore, antimicrobial prophylaxis as well as the prompt diagnosis of established infections is an important component in the management of HIV seropositive patients with AML.

In AML patients who successfully achieve remission, allogeneic stem cell transplantation is the most effective post-remission therapy in preventing future relapse of the disease (3, 42). In addition to maintaining remission, allogeneic stem cell transplantation from donors with a CCR5-d32 mutation to HIV seropositive AML recipients has been shown to potentially eradicate the HIV infection (43). Cysteine-cysteine chemokine receptor 5 (CCR5) is a cellular receptor that is required for HIV to enter and infect various human cells. A CCR5-d32 mutation, results in a defective receptor that is incapable of allowing HIV to effectively enter any cell that expresses the abnormal receptor. This CCR5-d32 mutation occurs with highest frequency among the European population. There are published case reports of two patients in Germany, in whom the HIV remains undetectable following an allogeneic stem cell transplant from donor stem cells with the CCR5-d32 mutation (42, 43). This has resulted in the discovery that homozygosity for the CCR5-d32 mutation (heterozygosity to a lesser extent), confers a degree of immunity to HIV infection and this immunity can be transferred to a previously infected patient.

The advent of HAART/cART has significantly improved the morbidity and mortality of HIV seropositive patients with malignancies, particularly Kaposi sarcoma and non-Hodgkin Lymphoma (29, 35). Patients should be initiated on combination antiretroviral therapy as soon as the HIV seropositivity is diagnosed, and this should be regardless of CD_4 count or clinical stage of disease, as a minimum standard of care

(34). Antiretroviral drugs, such as zidovudine, which have the potential to cause myelosuppression, are best avoided as part of combination antiretroviral therapy for patients with AML. Despite treatment with cART as well as chemotherapy, it has been shown that HIV seropositive patients with CD_4 counts below 200 cells/ul or clinically advanced disease have higher adverse outcomes and lower disease free survival rates (40, 41). This occurs mainly due to the overwhelming immunosuppression and prolonged neutropenia as a result of the natural history of HIV infection as well as the effects of chemotherapy (36, 40).

In the Clinical Haematology unit at CHBAH, there has been an increase in the number of patients with haematological malignancies who are concurrently infected with HIV. This includes AIDS-defining malignancies such as high grade, B-cell Non-Hodgkin Lymphoma, HIV-associated malignancies such as Hodgkin Lymphoma and malignancies that have a coincidental association with HIV infection (33, 34, 38). Currently, AML appears to be a coincidental haematological malignancy in the setting of HIV infection. In view of the paucity of information on HIV infection and AML in the South African population, this study was undertaken in order to evaluate the epidemiology, clinical presentation, treatment and outcomes of HIV seropositive patients with Acute Myeloid Leukaemia.

CHAPTER 2: PATIENTS AND METHODS

2.1. Aim

To describe the profile of patients diagnosed with Acute Myeloid Leukaemia (AML) in the Clinical Haematology unit at Chris Hani Baragwanath Academic Hospital (CHBAH), including those with coexistent HIV infection from 01/01/1993 to 31/12/2014.

2.2. Objectives

The objectives of this study are:

- To describe the demographics, clinical presentation, laboratory features and management of patients with AML, including HIV seropositive patients with AML from 01/01/2005 to 31/12/2014
- To describe the demographics, clinical presentation, laboratory features and management of HIV seropositive patients with AML from 01/01/1993 to 31/12/2004 in the era of cART not being available.

2.3. Methodology

2.3.1. Study design

A single center, retrospective, observational review of records was carried out of all adult patients with AML diagnosed in the Clinical Haematology unit at Chris Hani Baragwanath Academic hospital during the period 01/01/2005 to 31/12/2014 and for the HIV seropositive patients diagnosed with AML during the period 01/01/1993 to 31/12/2004.

2.3.2. Ethics

- Permission to conduct this study was obtained from the Heads of department for Clinical Haematology and Internal Medicine as well as from the hospital authorities at CHBAH.

- Ethics approval was obtained from the Human Resources Ethics Committee (HREC) at the University of the Witwatersrand, certificate number M141169.
- Patient records were reviewed retrospectively; therefore no consent was required.

2.3.3. Study population and setting

This study was conducted at Chris Hani Baragwanath Academic hospital, which is located in the city of Johannesburg, Gauteng province. CHBAH is a public sector, academic hospital that is affiliated to the University of the Witwatersrand. It is the third largest hospital in the world with approximately 3000 beds and serves over 150 000 inpatients and 500 000 outpatients every year as a referral center for the Southern Gauteng region. The Clinical Haematology unit sees and follows up approximately 400 new patients per year, and approximately 450 – 600 outpatients per month.

2.3.4. Inclusion criteria

- Patients ≥ 18 years of age, diagnosed with Acute Myeloid Leukaemia
- HIV seropositive and seronegative patients were included during the period 01/01/2005 to 31/12/2014 and HIV seropositive patients for the period 01/01/1993 to 31/12/2014.

2.3.5. Exclusion criteria

- Patients ≤ 18 years of age were excluded from the study.

2.3.6. Data collection:

A review of clinical records in the Clinical Haematology unit for the period 01/01/1993 to 31/12/2014 was performed. Data from patient files was reviewed and collected using a data collection sheet (see Appendix A). Data included demographics, clinical presentation, management, outcome and follow up. Laboratory investigations, including bone marrow findings, were recorded using a results flow chart (see Appendix B). Laboratory results were collected and reviewed at diagnosis of AML. The permission to access these clinical records was obtained from the relevant Heads of Department (Clinical Haematology and Internal Medicine) as well as the hospital administrative authorities at CHBAH.

2.3.7. Sampling

A total of 222 patients with AML were identified for the study period.

- For 20 patients, files and patient records could not be located. These patients were not included for analysis, leaving 202 patients.
- Seven patients were excluded as they were <18 years of age, leaving a total of 195 eligible patients.
- The remaining 195 patients included all AML patients seen during the period 01/01/2005 to 31/12/2014, together with 6 HIV seropositive AML patients from 01/01/1993 to 31/12/2004.

2.4. Statistical analysis

A sample size calculation was carried out in order to determine whether the population size was appropriate and to determine if the sample size would adequately address the objectives of the study. It was determined that a study population of 189 participants was appropriate for this research.

Regarding the descriptive data analysis, the categorical variables were summarized as percentage tabulation, frequencies and ratios. This data was represented by means of bar graphs and pie charts. The continuous variables were summarized by the mean and standard deviation, median and interquartile range, and their distribution illustrated by means of histograms.

Regarding the association between HIV status and the study variables, this was carried out as follows: the Chi^2 test was used to assess the relationship between HIV status and categorical variables, while Fisher's exact test was used for 2 x 2 tables or where the requirements for the Chi^2 test could not be met. The strength of the associations was measured by Cramer's V and the Phi coefficient respectively.

The relationship between continuous variables and HIV status was assessed by the t-test. Where the data did not meet the assumptions of these tests, a non-parametric alternative, the Wilcoxon rank sum test was used. The Cohen's d for parametric tests and the r-value for the non-parametric tests measured the strength of the associations.

Survival curves were derived using Kaplan-Meier estimation. Survival curves were compared using Cox proportional hazards regression. Data analysis was carried out using SAS software (Statistical Analysis System, version 9.4 for Windows). The 5% significance level was used, with a p-value of <0.05, indicating statistical significance.

A statistician was consulted for assistance with the statistical analysis.

2.5. Study significance

The management of HIV seropositive patients in the South African setting has become essential in clinical practice. As the survival of these patients improves with the advent of combination antiretroviral therapy, non-communicable diseases and malignancies in particular, are on the increase and are creating treatment challenges in the management of these patients. The prevalence of Acute Myeloid Leukaemia in association with HIV at the Clinical Haematology unit, CHBAH appears to be increasing. Although the association between HIV infection and AML is still regarded as being coincidental, recognition of this association has created a need for a study to be performed, to evaluate and to determine if there are any clinically significant differences in the presentation and outcomes of these patients compared to their HIV seronegative counterparts. This information could then be used to aid in the diagnosis and management of HIV seropositive patients with AML in the future and to plan further prospective studies to better characterize and manage this association.

CHAPTER 3: RESULTS

A total of 195 patients with Acute Myeloid Leukaemia treated at the Clinical Haematology unit at Chris Hani Baragwanath Academic Hospital and meeting the inclusion criteria were evaluated for the purpose of this study. One hundred and eighty nine patients with AML were evaluated for the 10-year study period 01/01/2005-31/12/2014.

The majority of patients with AML evaluated over this time period were HIV seronegative 162/189 (85.7%), and 27/189 (14.3%) were HIV seropositive.

A further 6 HIV seropositive patients with AML were evaluated for the study period 01/01/1993-31/12/2004 and have been included in this study as case reports.

3.1. Number of patients per year

The highest number of new AML patients that were seen in a single year was in 2014 (24 patients), and 23 new patients were seen each year in 2007, 2012 and 2013, respectively. Although a higher number of patients were seen in the years towards the latter part of the study period, a clear increasing trend in the number of AML cases seen is not conclusively demonstrated. The number of patients seen per year of the study period is summarized below in figure 3.1.

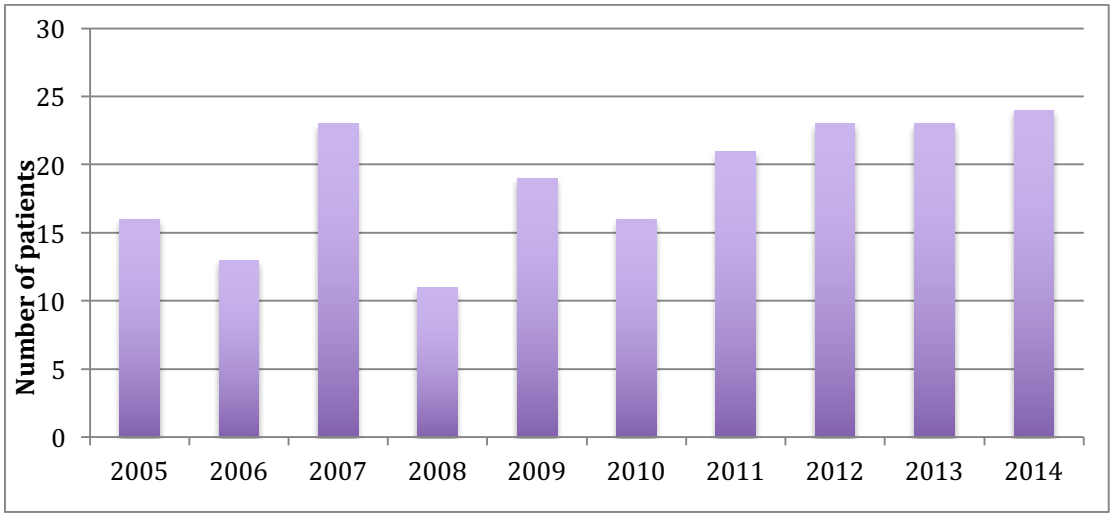


Figure 3.1: Number of patients by year of presentation

3.2. Demographics of Acute Myeloid Leukaemia

The majority of patients were of Black African ethnicity, i.e.173/189 (91.5%). There were 11/189 (5.8%) White, 1/189 (0.5%) Indian and 4/189 (2.1%) Coloured patients in the study population, respectively. There was an overall male predominance of 99/189 (52.4%) of the patients, compared to 90/189 (47.6%) of female patients, with a male: female ratio of 1.1:1. In the HIV seronegative AML group, there was a male predominance of 88/162(54.3%) of patients, compared to 74/162 (45.7%) of female patients. In the HIV seropositive group there was a female predominance of 16/27 (59.3%) of patients, compared to 11/27 (40.7%) of male patients, with a male: female ratio of 1:1.5 (see table 3.1 below).

The median age at presentation across the study was 45 years (range 18-88 years). In the subgroup analysis, the median age of the HIV seropositive AML group was 38 years (IQR 28-46years). This was significantly lower than that of the HIV seronegative AML group of 49 years (IQR 30-63 years) with a p-value of 0.027. The age distribution of patients in this study is summarized in table 3.1 below.

Table 3.1: Gender distribution of HIV seronegative compared to HIV seropositive AML patients

Variable	Overall N (%)	AML patients 2005-2014		p-value for between group t-test	All HIV seropositive patients 1993-2014
		HIV seronegative	HIV seropositive		
Number	189	162	27		33
Males	99/189 (52.4%)	88/162(54.3%)	11/27 (40.7%)	0.22	14/33 (42.4%)
Females	90/189 (47.6%)	74/162 (45.7%)	16/27 (59.3%)		19/33 (57.6%)
M:F ratio	1.1:1	1.2:1	1:1.5		1:1.4
Median age (*IQR)	45 years (18-88 years)	49 years (30-63 years)	38 years (28-46 years)	**0.027**	37 years (28-43 years)

*IQR= interquartile range

3.3. Presenting symptoms

Table 3.2: Presenting symptoms of AML patients

Symptoms	Overall N (%)	AML patients 2005-2014		p-value for between group test	All HIV seropositive patients 1993-2014
		HIV seronegative patients N=162	HIV seropositive patients N=27		N=33
Symptoms of anaemia	171 (92.4%)	148 (92.5%)	23 (92.0%)	0.67	29 (93.5%)
Symptoms of bleeding	96 (53.0%)	84 (53.8%)	12 (48.0%)	0.67	16 (53.3%)
Gums	49 (51.6%)	41 (49.4%)	8 (66.7%)	0.36	12 (75.0%)
Skin	47 (49.5%)	41 (49.4%)	6 (50.0%)	>0.99	6 (37.5%)
Epistaxis	40 (42.1%)	35 (42.2%)	5 (4.7%)	>0.99	7 (43.8%)
Malaena	12 (12.6%)	11 (13.3%)	1 (8.3%)	>0.99	2 (12.5%)
Menorrhagia	11 (11.6%)	8 (9.6%)	3 (25%)	0.14	4 (25%)
Haemoptysis	7 (7.4%)	6 (7.2%)	1 (8.3%)	>0.99	1 (6.3%)
Haematuria	4 (4.2%)	4 (4.8%)	0	>0.99	1 (6.3%)
Haematemesis	3 (3.2%)	3 (3.6%)	0	>0.99	1 (6.3%)
Symptoms of infection	53 (28%)	45 (27.8%)	8 (29.8%)	0.82	9 (27.3%)
Respiratory	37 (69.8%)	30 (66.7%)	7 (87.5%)	0.41	8 (88.9%)
Gastrointestinal	9 (17%)	8 (17.8%)	1 (12.5%)	>0.99	1 (11.1%)
Genitourinary	3 (5.7%)	3 (6.7%)	0	>0.99	0
Skin	2 (3.8%)	2 (4.4%)	0	>0.99	0
Musculoskeletal	2 (3.8%)	2 (4.4%)	0	>0.99	0
Central nervous system	2 (3.8%)	1 (2.2%)	0	>0.99	1 (11.1%)
Constitutional symptoms					
Weight loss	100 (52.9%)	84 (51.9%)	16 (59.3%)	0.54	19 (57.6%)
Fever	89 (47.1%)	72 (44.4%)	17 (63.0%)	0.096	21 (63.6%)
Night sweats	87 (46.0%)	72 (44.4%)	15 (55.6%)	0.30	18 (54.5%)
Bone pain	29 (16.6%)	25 (16.6%)	4 (16.7%)	>0.99	5 (16.7%)

The most common presenting complaints were related to symptoms of anaemia, which were found in 92.4% of patients. Bleeding manifestations were reported in 53.0% of patients. In the patients presenting with bleeding, the commonest site was gum bleeding, found in 51.6 % of patients. Symptoms of infection were present in 28.0% of the patients at presentation, and the commonest site of infection was the respiratory tract, in 69.8% of patients. There was no significant difference with regard to sites of bleeding or symptoms of infection between the HIV seronegative and the HIV seropositive patients.

In patients who reported the presence of constitutional symptoms, the commonest symptom was of loss of weight in 52.9% of patients. Fever and night sweats were reported 47.1% and 46.0% of patients, respectively. Bone pain was an uncommon symptom, being present in only 16% of patients. The presenting clinical features on history are summarized in table 3.2 above.

3.4. Exposure to risk factors for AML

A total of 7 (3.7%) AML patients in the study (6 HIV seronegative and 1 HIV seropositive) had a previously documented malignancy. All of them received chemotherapy for a prior primary malignancy. Occupational exposure to potentially leukaemogenic agents was found in 10 patients; 8 of these patients were HIV seronegative and 2 were HIV seropositive. Among those with occupational exposures, 5 patients worked in the mining industry while the remaining 5 patients had exposure to chemicals in the dry cleaning, fertilizer production, chemical engineering, petroleum and aluminum production industries.

3.5. HIV seropositive patients with AML

During the 10-year study period 01/01/2005-31/12/2014, a total of 27 HIV seropositive patients were diagnosed with AML at Chris Hani Baragwanath Academic Hospital. Among these patients, 40% were diagnosed with HIV prior to the diagnosis of AML, while in 60% of the patients; the diagnosis of HIV was made simultaneously with the diagnosis of AML. In the patients with an antecedent diagnosis of HIV, all the patients were on treatment with cART and the median time difference between the diagnosis of HIV and the diagnosis of AML was 1.4 years (range of 3 months to 7 years). The mean CD_4 count at presentation was 353 cells/ul (range of 29-1379 cells/ul). This included HIV seropositive patients who were on treatment with cART and those who were treatment naïve.

The majority of HIV seropositive patients in this study were classified as WHO clinical stage 1, at the time of being diagnosed with AML. The WHO clinical staging for the HIV seropositive patients is summarized in figure 3.2 below.

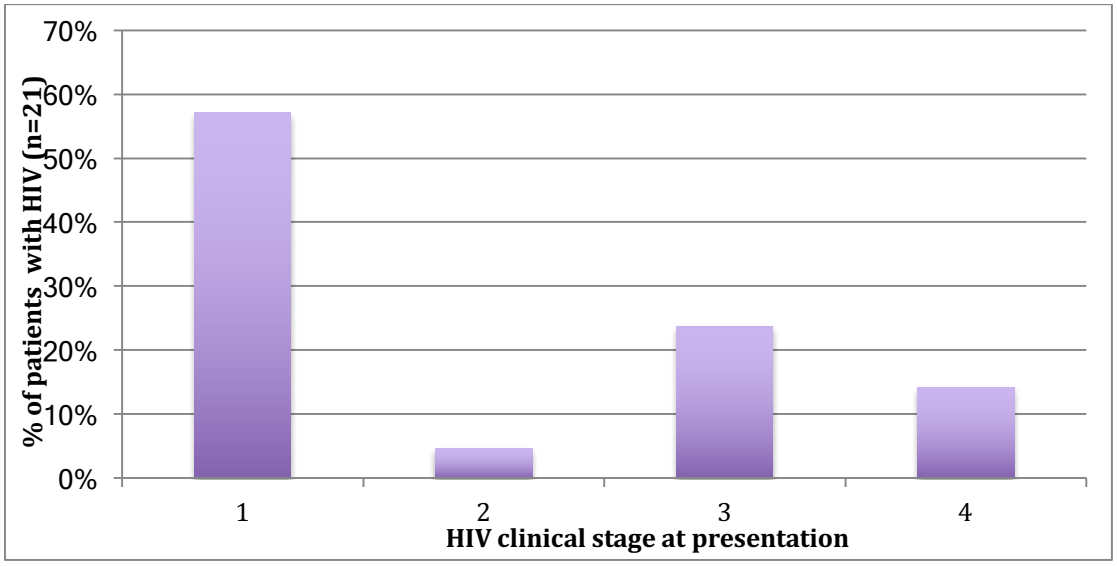


Figure 3.2: The distribution of HIV seropositive patients by WHO (World Health Organization) clinical stage

The presence of disseminated tuberculosis (TB) was the commonest clinical criteria for the patients who were categorized into WHO clinical stage 4. All patients who had a preceding diagnosis of HIV were on combination antiretroviral therapy (cART). Only one of these patients had previously required a change in the drug regimen due to an adverse drug effect. Among the patients that were on cART, the median duration on treatment was 9 months (range of 0.7-84 months).

3.6. Clinical signs

Table 3.3: Presenting clinical signs of AML patients

Clinical signs	Overall N (%)	AML patients 2005-2014		p-value for between group t-test	All HIV seropositive patients 1993-2014
		HIV seronegative N=162	HIV seropositive N=27		N=33
Pallor	178 (94.2%)	153 (94.4%)	25 (92.6%)	0.66	31 (93.9%)
Lymphadenopathy	61 (32.3%)	51 (31.5%)	10 (37%)	0.66	12 (36.4%)
Signs of bleeding					
Ecchymosis	68 (36.0%)	59 (36.4%)	9 (33.3%)	0.83	11 (33.3%)
Petechiae	43 (22.8%)	39 (24.1%)	4 (14.8%)	0.33	7 (21.2%)
Purpura	43 (22.8%)	40 (24.7%)	3 (11.1%)	0.14	5 (15.2%)
Haemorrhagic bullae	11 (5.8%)	11 (6.8%)	0	0.37	0
Evidence of infection					
Respiratory	36 (67.9%)	27 (62.8%)	9 (90.0%)	0.14	11 (91.7%)
Gastrointestinal	3 (5.7%)	3 (7.0%)	0	>0.99	0
Genitourinary	8 (15.1%)	7 (16.3%)	1 (10%)	>0.99	1 (8.3%)
Skin	4 (7.5%)	4 (9.3%)	0	>0.99	0
Musculoskeletal	2 (3.8%)	2 (4.7%)	0	>0.99	0
Central nervous system	4 (7.5%)	3 (7.0%)	0	>0.99	1 (9.0%)
Tuberculosis	16 (8.5%)	9 (5.6%)	7 (26.0%)	**0.0018**	7 (22.6%)
Organomegaly					
Hepatomegaly	77 (40.7%)	66 (40.7%)	11 (40.7%)	>0.99	13 (39.4%)
Splenomegaly	48 (25.4%)	42 (25.9%)	6 (22.2%)	0.81	6 (18.2%)
Abdominal mass	6 (3.2%)	5 (3.1%)	1 (3.7%)	>0.99	2 (6.1%)
Extramedullary					
Gum hypertrophy	22 (11.6%)	20 (12.3%)	2 (7.4%)	0.75	3 (9.1%)
Myeloid sarcoma	13 (6.9%)	10 (6.2%)	3 (11.1%)	0.40	3 (9.1%)
Skin	5 (2.6%)	4 (2.5%)	1 (3.7%)	0.54	2 (6.1%)

On general examination, 94% of the HIV seronegative AML patients were found to have pallor, with a similar percentage (92.6%) of HIV seropositive patients manifesting with pallor. Lymphadenopathy was found in 32% of the overall population, the proportion of patients with lymphadenopathy was similar in the HIV seronegative and HIV seropositive AML patients at 31.5% and 37.0%, respectively.

The most common clinically evident type of cutaneous bleeding seen was ecchymosis seen in 36.4% of HIV seronegative AML patients and 33.3% of HIV seropositive AML patients. Petechiae were present in 24.1% of HIV seronegative AML patients

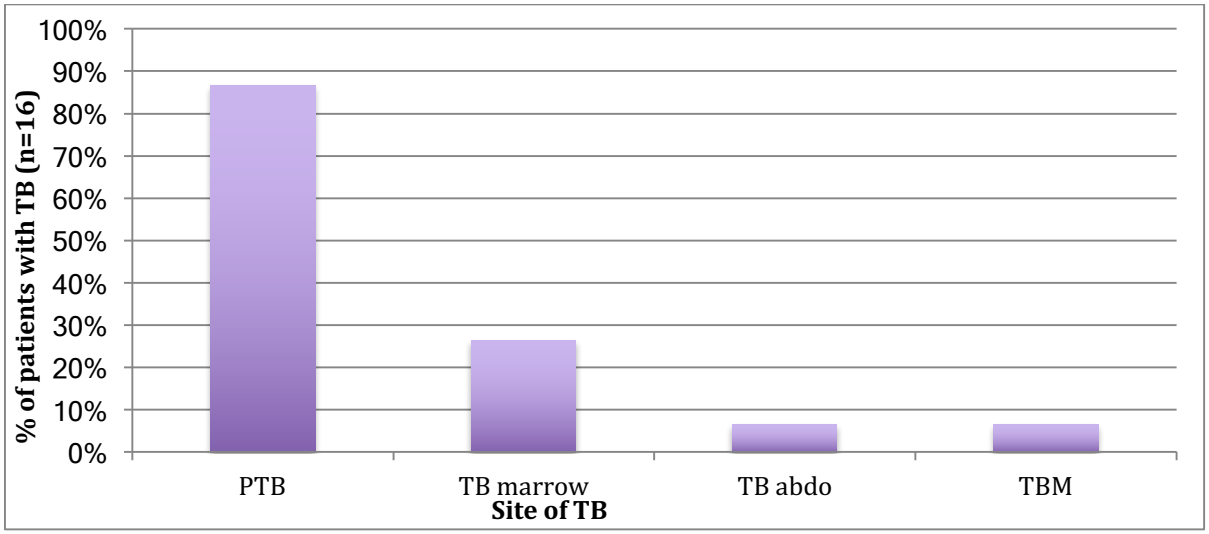
and 14.1% of HIV seropositive patients. No significant difference in the site of bleeding was found between the two study populations.

In this study, 53 patients representing 28.8% of the total study population had a clinically evident infection. Within these 53 patients, the most common site of infection was the respiratory tract (67.9%). Among these patients with clinically evident infection, only 6/53 (11.3%) had positive blood cultures. The most common organisms that were cultured were gram-negative bacilli, present in 5 out of the 6 patients. In the patients with positive blood cultures: 2 patients cultured Klebsiella Pneumonia, 2 patients cultured Escherichia Coli, 1 patient cultured Pseudomonas Aeruginosa and 1 patient cultured Nocardia species.

The presenting clinical signs of patients with AML are summarized in table 3.3.

3.6.1. Presence of Tuberculosis

Tuberculosis was present in 16/189 (8.5%) of the patients. The proportion of patients with TB was higher in the HIV seropositive group 7/27 (26.0%) compared to the HIV seronegative group 9/162 (5.6%). This difference was statistically significant (p=0.0018). Of the 16 patients with TB, the site of TB infection is shown below in figure 3.3. In 4 patients, the diagnosis of TB was made on histology of bone marrow samples that were evaluated for AML. Some patients had more than one site of TB, hence the percentages do not add up to 100%. The most common site and type of TB was pulmonary TB, in 86.7% of the patients.



PTB=Pulmonary tuberculosis; TB abdo= Abdominal tuberculosis; TBM= Tuberculous meningitis.

Figure 3.3: Tuberculosis in patients with AML

3.6.2. Organomegaly

The presence of hepatomegaly was found in 77/189 (40.7%) of the overall population. This was represented by 66/162 (40.7%) of HIV seronegative AML patients and 11/27 (40.7%)HIV seropositive AML patients. The proportions of patients with hepatomegaly were similar in both study populations. Splenomegaly was found in 48/189 (25.4%) of the overall population; 42/162 (25.9%) in HIV seronegative patients and 6/27 (22.2%) in HIV seropositive patients. An abdominal mass was detected in 6/189 (3.2%) of the patients, with 5/162 (3.1%) being found in HIV seronegative and only 1/27 (3.7%) of the HIV seropositive patients. A summary of organomegaly is provided in figure 3.4 below.

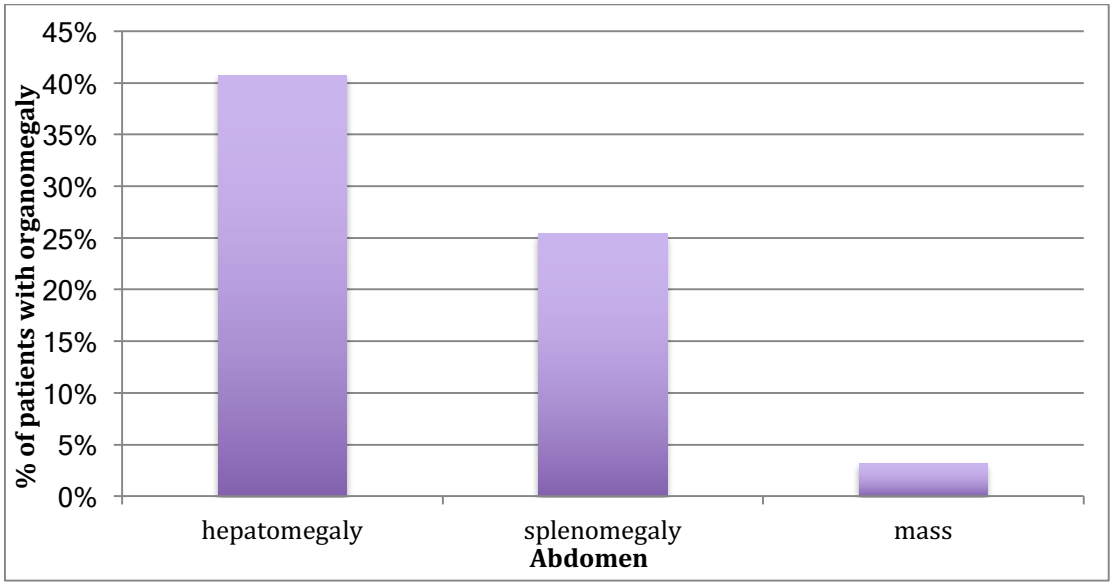


Figure 3.4: Organomegaly and abdominal masses in AML patients.

3.6.3. Extramedullary disease

The overall incidence of extramedullary disease in this population of AML patients was 40/189 (21.2%). Of these patients 34/40 (85.0%) were HIV seronegative, comprising 20.9% of the total HIV seronegative AML group. A further 6/40 (15.0%) were HIV seropositive, comprising 22.2% of the HIV seropositive AML group. The most common site of extramedullary disease was gum hypertrophy in 22/40 (55.0%) of patients; 20 of these patients were HIV seronegative and 2 patients were HIV seropositive. A total of 13 patients presented with a myeloid sarcoma; 10 of these patients were HIV seronegative and 3 were HIV seropositive. The overall incidence of myeloid sarcomas in this study population was found to be 6.9%. There was no statistically significant difference in the presence of extramedullary disease between the HIV seronegative and HIV seropositive AML patients. A summary of the extramedullary manifestations of AML are provided in figure 3.5 below.

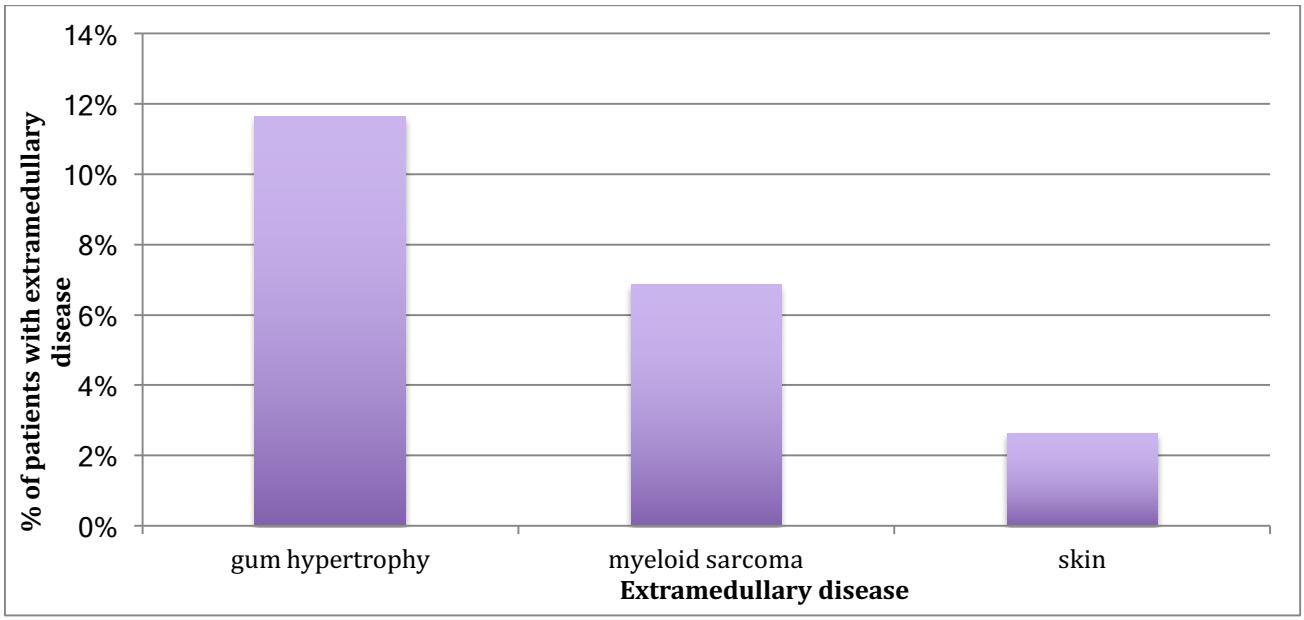


Figure 3.5: Extramedullary manifestations of AML

3.7. De novo (primary) and secondary AML

In this study, the majority of patients with AML, i.e. 132/169 (69.8%) had de novo AML. The overall prevalence of secondary AML in this cohort was 57/189 (30.2%). Of these patients, 54 were HIV seronegative. Among the 3 HIV seropositive patients with secondary AML, 1 patient had chemotherapy related AML and 2 patients had preceding myelodysplasia. Among the 54 HIV seronegative patients with secondary AML, the most common pathogenesis was a preceding myelodysplastic process with a frequency of 26/54 (48.1%), followed by a preceding myeloproliferative disorder in 21/54 (38.9%). Chemotherapy related AML accounted for 6/54 (11.1%) of the secondary AML patients and 1/54 (1.9%) of secondary AML patients evolved from a prior diagnosis of aplastic anaemia. A summary of the aetiology of secondary AML in this study is provided in table 3.4 below.

Table 3.4: De novo (primary) and secondary AML

Primary or secondary AML	Overall N=189	HIV seronegative N=162	HIV seropositive N=27
Primary	132 (69.8%)	108 (66.7%)	24 (88.9%)
Secondary	57 (30.2%)	54 (33.3%)	3 (11.1%)
Myelodysplasia		26 (48.1%)	2 (7.4%)
Myeloproliferative disorder		21 (38.9%	0
Chemotherapy related		6 (11.1%)	1 (3.7%)
Aplastic anaemia		1 (1.8%)	0

3.8. Laboratory parameters of patients with Acute Myeloid Leukaemia

Table 3.5: Laboratory parameters of patients with AML

Variable	Category	Overall		AML patients 2005-2014				p-value for between-group test	HIV seropositive 1993-2014	
				HIV seronegative		HIV seropositive				
		N=189	%	N=162	%	N=27	%		N=33	%
WCC x10^9/l (n=188)	<4	56	29.8%	51	31.7%	5	18.5%	0.17	7	21.2%
	4-11	40	21.3%	31	19.3%	9	33.3%		11	33.3%
	>11	92	48.9%	79	49.1%	13	48.1%		15	45.5%
Haemoglobin g/dl (n=188)	Hb <10	168	89.4%	143	88.8%	25	92.6%	0.74	30	90.9%
	Females < 12 (n=90)	90	100.0%	74	100.0%	16	100.0%	-	18	94.7%
	Males <14 (n=98)	97	99.0%	86	98.9%	11	100.0%	>0.99	14	100.0%
Platelets x10^9/l (n=188)	<150	164	87.2%	140	87.0%	24	88.9%	0.77	29	87.9%
	150-450	19	10.1%	17	10.6%	2	7.4%		3	9.1%
	>450	5	2.7%	4	2.5%	1	3.7%		1	3.0%
MCV fl (n=181)	<80	15	8.3%	13	8.3%	2	8.0%	0.33	2	6.5%
	80-100	122	67.4%	108	69.2%	14	56.0%		19	61.3%
	>100	44	24.3%	35	22.4%	9	36.0%		10	32.3%
Sodium mmol/l (n=186)	<135	61	32.8%	45	28.1%	16	61.5%	**0.0033**	19	59.4%
	135-145	116	62.4%	107	66.9%	9	34.6%		12	37.5%
	>145	9	4.8%	8	5.0%	1	3.8%		1	3.1%
Potassium mmol/l (n=186)	<3.5	36	19.4%	31	19.4%	5	19.2%	>0.99	7	10.9%
	3.5-5.3	146	78.5%	125	78.1%	21	80.8%		25	39.1%
	>5.3	4	2.2%	4	2.5%	0	0.0%		32	50.0%

Variable	Category	Overall		AML patients 2005-2014				p-value for between-group test	HIV seropositive 1993-2014	
				HIV seronegative		HIV seropositive				
		N=189	%	N=162	%	N=27	%		N=33	%
Calcium mmol/l (n=158)	<2.05	14	8.9%	13	9.6%	1	4.5%	0.47	1	4.2%
	2.05-2.56	140	88.6%	120	88.2%	20	90.9%		22	91.7%
	>2.56	4	2.5%	3	2.2%	1	4.5%		1	4.2%
Phosphate mmol/l (n=158)	0.8-1.4	111	70.3%	102	75.0%	9	40.9%		10	41.7%
	>1.4	29	18.4%	18	13.2%	11	50.0%		12	50.0%
Albumin g/l (n=170)	<35	74	43.5%	60	41.1%	14	58.3%	0.25	17	56.7%
	35-50	95	55.9%	85	58.2%	10	41.7%		13	43.3%
	>50	1	0.6%	1	0.7%	0	0.0%		0	0.0%
Ferritin ng/ml (n=147)	<15	1	0.7%	1	0.8%	0	0.0%	**0.034**	0	-
	15-300	19	12.9%	13	10.2%	6	31.6%		7	36.8%
	>300	127	86.4%	114	89.1%	13	68.4%		13	68.4%
Vitamin B12 pmol/l (n=136)	<145	8	5.9%	6	5.1%	2	10.5%	0.30	2	10.0%
	145-637	56	41.2%	51	43.6%	5	26.3%		5	25.0%
	>637	72	52.9%	60	51.3%	12	63.2%		13	65.0%
Urea mmol/l (n=186)	< 2.0	4	2.2%	3	1.9%	1	3.8%	0.65	1	3.1%
	2.0-7.0	130	69.9%	112	70.0%	18	69.2%		22	68.8%
	>7.0	52	28.0%	45	28.1%	7	26.9%		9	28.1%
Creatinine umol/l (n=168)	40-100	164	97.6%	143	99.3%	21	87.5%	**0.0094**	26	89.7%
	>100	4	2.4%	1	0.7%	3	12.5%		3	10.3%
Total Bili umol/l (n=168)	0-21	152	90.5%	132	91.7%	20	83.3%	0.25	25	83.3%
	>21	16	9.5%	12	8.3%	4	16.7%		5	16.7%

Variable	Category	Overall		AML patients 2005-2014				p-value for between-group test	HIV seropositive 1993-2014	
				HIV seronegative		HIV seropositive				
		N=189	%	N=162	%	N=27	%		N=33	%
ALP U/l (n=167)	0-120	135	80.8%	114	79.7%	21	87.5%	0.58	26	86.7%
	>120	32	19.2%	29	20.3%	3	12.5%		4	13.3%
GGT U/l (n=167)	0-50	92	55.1%	74	51.7%	18	75.0%	**0.045**	24	80.0%
	>50	75	44.9%	69	48.3%	6	25.0%		6	20.0%
AST U/l (n=167)	0-40	131	78.4%	113	79.0%	18	75.0%	0.60	24	80.0%
	>40	36	21.6%	30	21.0%	6	25.0%		6	20.0%
ALT U/l (n=167)	0-40	140	83.8%	119	83.2%	21	87.5%	0.77	27	90.0%
	>40	27	16.2%	24	16.8%	3	12.5%		3	10.0%
Uric acid mmol/l (n=105)	<0.2	14	13.3%	12	11.4%	2	1.9%	0.62	2	12.5%
	0.2-0.35	36	34.3%	32	30.5%	4	3.8%		4	25.0%
	>0.35	55	52.4%	47	44.8%	8	7.6%		10	62.5%
LDH U/l (n=113)	100-200	7	6.2%	7	6.2%	0	-	-	0	-
	>200	106	93.8%	89	78.8%	17	15.0%		18	100.0%

WCC= white cell count; MCV=mean cell volume; Total Bili= total bilirubin; ALP= alkaline phosphatase; GGT= gamma-glutamyl transferase; AST= aspartate aminotransferase; ALT= alanine aminotransferase; LDH= lactate dehydrogenase.

Regarding the laboratory parameters, the HIV seropositive AML patients had lower mean haemoglobin, platelet and albumin levels, compared to their HIV seronegative counterparts. These differences, however, were not statistically significant. The HIV seropositive AML patients were noted to have statistically significantly lower serum sodium (Na) and gamma glutamyl transferase (δGT) levels, compared to the HIV seronegative patients. The HIV seronegative group had higher levels of ferritin with significantly more HIV seronegative patients with ferritin levels above 300 ng/ml; this difference was statistically significant, with a p-value of 0.034. The HIV seropositive patients were more likely to have a higher serum creatinine than the HIV seronegative AML patients; this difference was statistically significant, with a p-value of 0.0094. Similar laboratory values were seen between the two groups for the following parameters: potassium, calcium, phosphate, vitamin B12, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. A summary of the laboratory findings in this study are tabulated in table 3.5 above.

Regarding the HIV seropositive patients with AML, the mean CD_4 count at the time of the AML diagnosis was 353 cells/ul, with a range of 29-1379 cells/ul. There were 11 patients (47.8%) with CD_4 counts below 200 cells/ul and a smaller number of 3 patients (13%) who had a CD_4 count below 50 cells/ul. The CD_4 count findings are tabulated in table 3.6 below.

Table 3.6: CD_4 counts in HIV seropositive patients with AML

Variable	Category	HIV seropositive patients N=23	HIV seropositive patients on cART N=9	HIV seropositive patients cART naïve N=14
CD_4 cell count cells/ul	>501	5 (21.7%)	2 (22.2%)	3 (21.4%)
	351-500	5 (21.7%)	2 (22.2%)	3 21.4%)
	201-350	2 (8.7%)	2 (22.2%)	0
	101-200	8 (34.8%)	1 (11.1%)	7 (50%)
	50-100	0	0	0
	<50	3 (13.0%)	2(22.2%)	1 (7.1%)

With regard to the HIV seropositive patients with AML, the mean viral load was 101 935 RNA copies/ml. There were 4 patients who were virologically suppressed; with 2 patients having HIV viral loads that were undetectable and a further 2 patients with HIV viral loads of 25 and 45 copies/ml, respectively. The HIV viral load results are tabulated in table 3.7 below.

Table 3.7: HIV viral load in seropositive patients with AML

HIV seropositive AML	Total number	N	Minimum	Maximum	Mean	Standard deviation
HIVVL copies/ml	27	9	0	638 497	101 935	±207 286

3.9. Bone marrow assessment

3.9.1. Bone marrow morphology: cellularity

The marrow was hypercellular in 56.5% of patients, normocellular in 31.7% of patients and hypocellular in 3.1% of patients. The "other" category (8%), represents the proportion of samples in which cellularity could not be accurately assessed. The marrow cellularity is represented by category in figure 3.6 below.

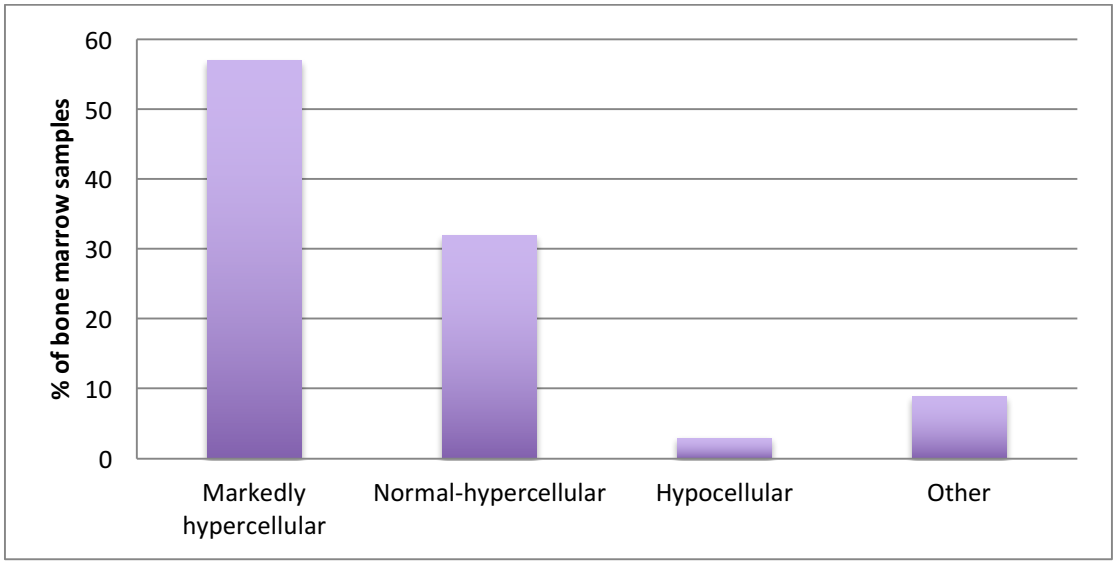


Figure 3.6: Bone marrow morphology by cellularity

Myelodysplasia was noted in 30.2% of HIV seronegative and 25.9% of HIV seropositive AML patients. There was no significant difference in the presence of myelodysplasia in the bone marrow samples of these two patient groups.

3.9.2. Bone marrow morphology: Histological subtype by French-American-British classification

Table 3.8: Frequency of AML histological subtypes

FAB histological subtype	Overall N=129	HIV seronegative N=108	HIV seropositive N=21	ALL HIV seropositive N=26
M0	14 (10.9%)	12 (11.1%)	2 (9.5%)	2 (7.7%)
M1	10 (7.8%)	10 (9.3%)	0	1 (3.8%)
M2	31 (24.0%)	21 (19.4%)	10 (47.6%)	11 (42.3%)
M3	29 (22.5%)	22 (20.4%)	7 (33.3%)	10 (38.5%)
M4	22 (17.1%)	20 (18.5%)	2 (9.5%)	2 (7.7%)
M5	14 (10.9%)	14 (12.9%)	0	0
M6	1 (0.8%)	1 (0.9%)	0	0
M7	8 (6.2%)	8 (7.4)	0	0

The most common histological subtype of AML, found in both the HIV seronegative and HIV seropositive groups is AML M2 (myeloblastic with granulocytic maturation), in 25% of the patients, while the least common subtype of AML encountered was AML M6 (erythroleukaemia). The AML M3 subtype was the second most common histological subtype across both study groups affecting 23% of the overall population. The AML M2 and M3 subtypes occurred at a higher frequency among the HIV seropositive AML patients at 45% and 40% respectively. Subtypes M0 and M4 occurred with higher frequency among the HIV seronegative AML group. The frequency of each histological subtype in the HIV seronegative and HIV seropositive AML patients are represented in table 3.8 above.

3.9.3. Bone marrow cytogenetics

3.9.3.1. Favourable cytogenetics

The most common cytogenetic abnormality with a favourable prognosis that was found in this study population is translocation (15;17), in association with a positive PML-RARA gene. This was present in 97% (28/29) of the patients with AML M3 subtype, and represents 16.8% of the total study population with documented cytogenetic results. In the sub-group analysis 20 out of 28 AML M3 patients with t(15;17) were HIV seronegative, the prevalence of t(15;17) in the HIV seronegative patients with AML is 14%. Eight out of 28 AML M3 patients with t(15;17) were HIV seropositive. The prevalence of t (15;17) in HIV seropositive patients with AML is 33%. This difference was statistically significant with a p value =0.0034.

Translocation (8;21), is associated with the RUNX1/RUNX1T1 gene. It was found in 77% (24/31) of the patients with AML M2 subtype, representing 14.4 % of the total study population with documented cytogenetic results. In the sub-group analysis 16 out of 24 AML M2 patients with t(8;21) were HIV seronegative. The prevalence of t(8;21) among HIV seronegative patients with AML is 11.2%. In the HIV seropositive group 8 out of 24 patients with AML M2 had t(8;21). Therefore, the prevalence of t(8;21) among HIV seropositive patients with AML is higher at 33.3%. This difference is statistically significant with a p value =0.0091. Inversion (16) was the least common favourable cytogenetic abnormality found in only 2.4% of the patients, and all of these patients were HIV seronegative.

3.9.3.2. Unfavorable cytogenetics:

The most common unfavourable cytogenetic abnormality found in this study population was translocation (9;22), which is associated with the BCR-ABL gene. A deletion abnormality in chromosome 7 (7q-) occurred in 6.0% of patients. The least common finding was a deletion of the long arm of chromosome 5 (5q-), with a prevalence of 2.4% of the overall population. A summary of the cytogenetic abnormalities found in the AML patients is tabulated in table 3.9 below.

Table 3.9: Frequency of favourable and unfavourable cytogenetic abnormalities in AML

Cytogenetics		Overall		HIV				p-value for between group test
				seronegative		seropositive		
		N	%	N	%	N	%	
		189		162		27		
Favourable	t(15;17)	28	16.8%	20	14%	8	33%	**0.034**
	t(8;21)	24	14.4%	16	11%	8	33%	**0.0091**
	inv (16)	4	2.4%	4	2.8%	0	0%	>0.99
Unfavourable	t(9;22)	11	6.6%	11	7.7%	0	0%	0.37
	7q-	10	6.0%	10	7.0%	0	0%	0.36
	5q-	4	2.4%	4	2.8%	0	0%	>0.99

3.10. Treatment

3.10.1. Induction chemotherapy

In this study 81.9% of patients received induction chemotherapy. The most common regimen administered was the "3+7" regimen, which consists of a combination of an anthracycline (daunorubicin) for three (3) days with cytosine arabinoside for seven (7) days. This regimen was administered to 60% of patients as induction chemotherapy. In 10.2% of patients the cytosine arabinoside component of induction was reduced to only five (5) days and combined with the standard anthracycline for three days, while 12.2% of patients received cytosine arabinoside as a single agent. All-transretinoic acid (ATRA) combined with an anthracycline (daunorubicin) was administered in 15% of patients, who had AML M3 (acute promyelocytic leukaemia). Drug regimens that were administered for induction chemotherapy in this study are summarized in figure 3.7 below.

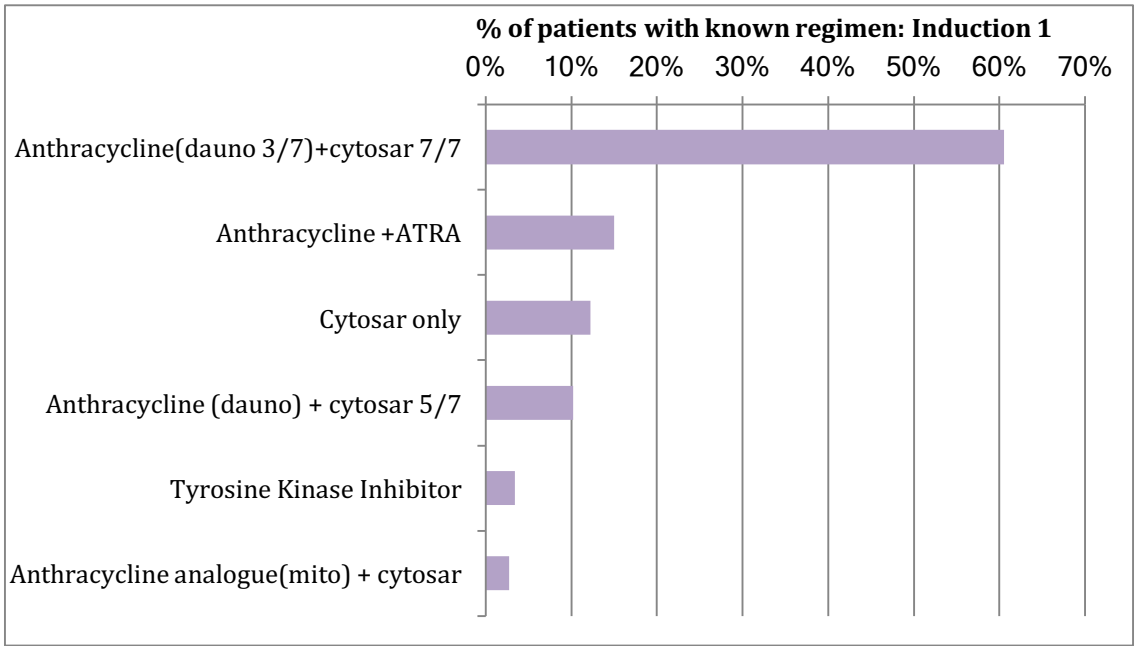


Figure 3.7: Chemotherapy regimens administered during induction therapy in AML patients

There was a lower percentage of HIV seropositive patients (35.0%) on the anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 7 days regimen, compared to HIV seronegative patients (64.6%). These findings were statistically significant (Fisher's exact test; p value=0.015).

Similarly, there was a higher percentage of HIV seropositive patients (25.0%) on the anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 5 days regimen, compared to HIV seronegative patients (7.7%). This difference was statistically significant (Fisher's exact test; p value=0.034).

3.10.2. Reinduction cycle 1

During the course of their treatment, 48.3% of patients required a reinduction cycle of chemotherapy due to either primary or secondary refractory disease. Of these patients, the majority (57.1%) were treated with the combination of mitoxantrone, cytosine arabinoside and etoposide. A further 17% of patients received a combination of an anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 7 days, 8.6% of patients received a combination of daunorubicin and cytosine arabinoside and etoposide. A summary of the drug regimens used in reinduction cycle 1 is shown in figure 3.8 below.

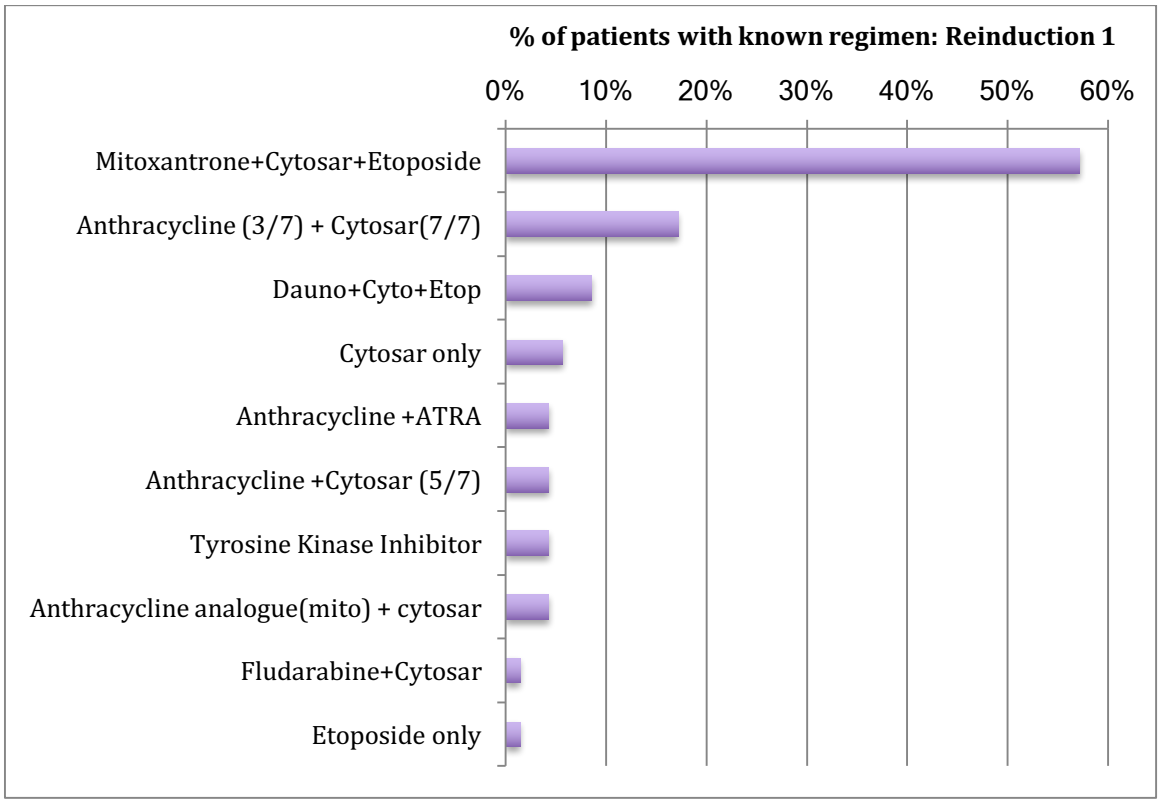


Figure 3.8: Chemotherapy regimens administered during reinduction cycle 1 in AML patients

There were no significant differences in the chemotherapy regimens administered for the first cycle of reinduction between the HIV seronegative and HIV seropositive patients.

3.10.3. Reinduction cycle 2

A total of 50% of the patients, who received reinduction cycle 1, failed to achieve remission and proceeded to reinduction cycle 2. In this group of patients the most common drug regimen administered in 54.3% of the patients, was a combination of mitoxantrone, cytosine arabinoside and etoposide. A further 17% of patients were treated with cytosine arabinoside as a single agent and 14% of patients received a combination of fludarabine and cytosine arabinoside. The standard combination of an anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 7 days was given to a minority of 8.6% of patients. A summary of the drug regimens administered during reinduction cycle 2 is shown in figure 3.9 below.

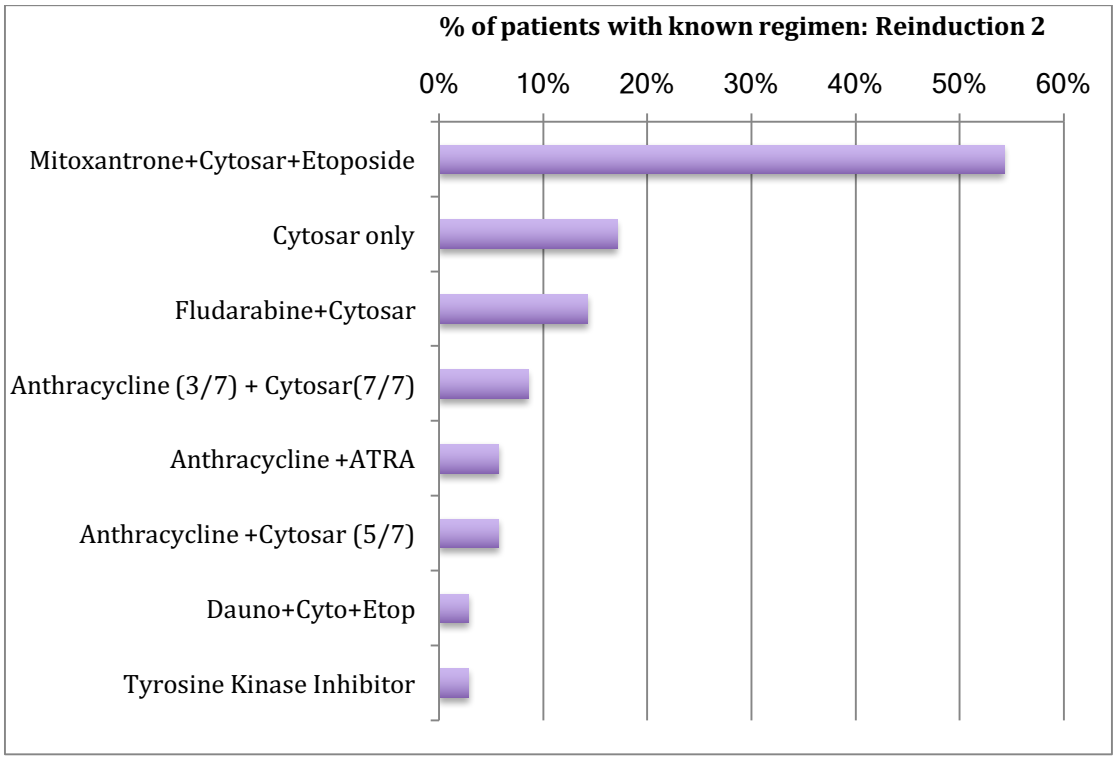


Figure 3.9: Chemotherapy regimens administered during reinduction cycle 2 in AML patients

In this group, the patients who received the standard anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 7 days regimen were HIV seronegative (8.6%),

while all the patients who received the anthracycline (daunorubicin) for 3 days and cytosine arabinoside for 5 days regimen during reinduction cycle 2, were HIV seropositive (5.7%). This difference was statistically significant with a p value of 0.033.

3.10.4. Reinduction cycle 3

A total of 64% of patients who had received reinduction cycle 2 went on to require a third cycle of induction chemotherapy during their course of treatment. In this group, an equal number received a combination of mitoxantrone, cytosine arabinoside and etoposide, to those who were given a combination of fludarabine and cytosine arabinoside, both with a frequency of 38%. A further 10% of patients were given cytosine arabinoside as single agent chemotherapy. A single patient each (4.8%) received daunorubicin and cytosine arabinoside for 5 days and mitoxantrone and cytosine arabinoside respectively.

There were no differences in regimens given between the HIV seropositive and the HIV seronegative patients with AML for this cycle of chemotherapy. A summary of drug regimens given for this cycle of chemotherapy is shown in figure 3.10 below.

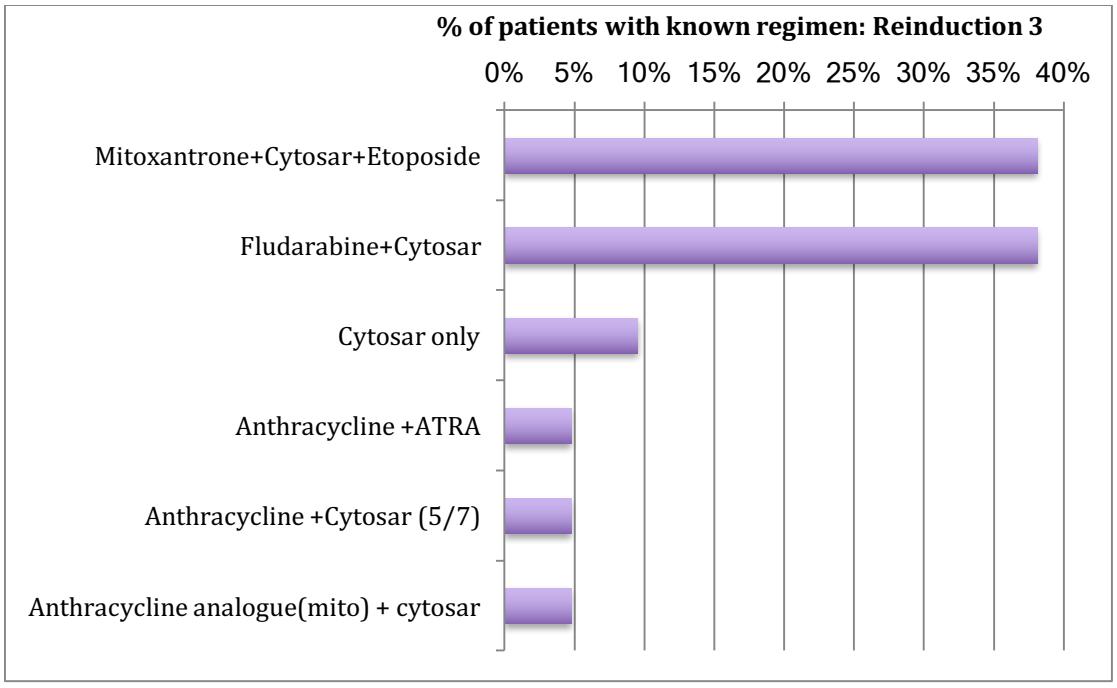


Figure 3.10: Chemotherapy regimens administered during reinduction cycle 3 in AML patients

3.10.5. Reinduction cycle 4

A further 32% of patients who had received reinduction cycle 3, went on to receive a fourth cycle of reinduction therapy during their course of treatment. This group of patients represented 4 % (7/189) of the total study population. Among these patients, 5 received a combination of fludarabine and cytosine arabinoside. One patient was given a combination of mitoxantrone, cytosine arabinoside and etoposide and the last patient received a combination of daunorubicin, cytosine arabinoside and etoposide.

Only 2 patients received further reinduction cycles 5 and 6. Both of them were HIV seronegative. One patient was given a course of azacytidine for both these cycles of chemotherapy. The other patient received fludarabine and cytosine arabinoside then cytosine arabinoside as a single agent for reinduction cycles 5 and 6, respectively.

3.10.6. Consolidation chemotherapy

Consolidation cycle 1

In this study, 64.8% of patients who achieved remission received consolidation chemotherapy. The most common regimen given for the first cycle of consolidation chemotherapy was a combination of an anthracycline (daunorubicin) and cytosine arabinoside at a high dose, administered 12 hourly, intravenously, for three (3) days. There were no significant differences in the chemotherapy regimens administered for the first cycle of consolidation therapy between the HIV seronegative and HIV seropositive patients. The frequency of regimens given for the first cycle of consolidation chemotherapy is summarized in figure 3.11 below.

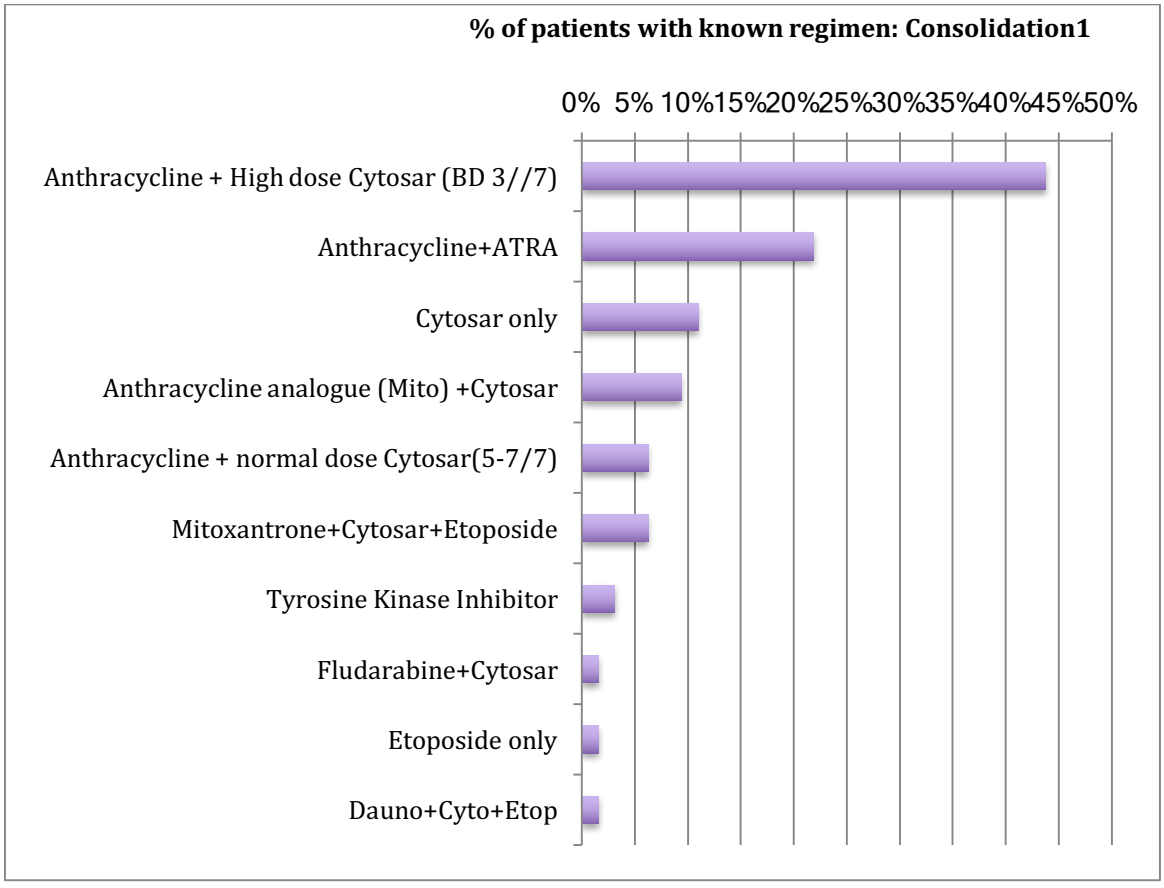


Figure 3.11: Chemotherapy regimens administered during consolidation cycle 1 in AML patients

Consolidation cycle 2

Approximately 84% of patients who were successfully treated with the first cycle of consolidation chemotherapy proceeded to receive a second cycle of consolidation chemotherapy. The most common regimen administered for this cycle of chemotherapy was a combination of an anthracycline (daunorubicin) and high-dose cytosine arabinoside. There were no significant differences in the chemotherapy regimens administered during the second cycle of consolidation between the HIV seronegative and the HIV seropositive patients. The frequency of regimens administered for the second cycle of consolidation chemotherapy are summarized in figure 3.12 below.

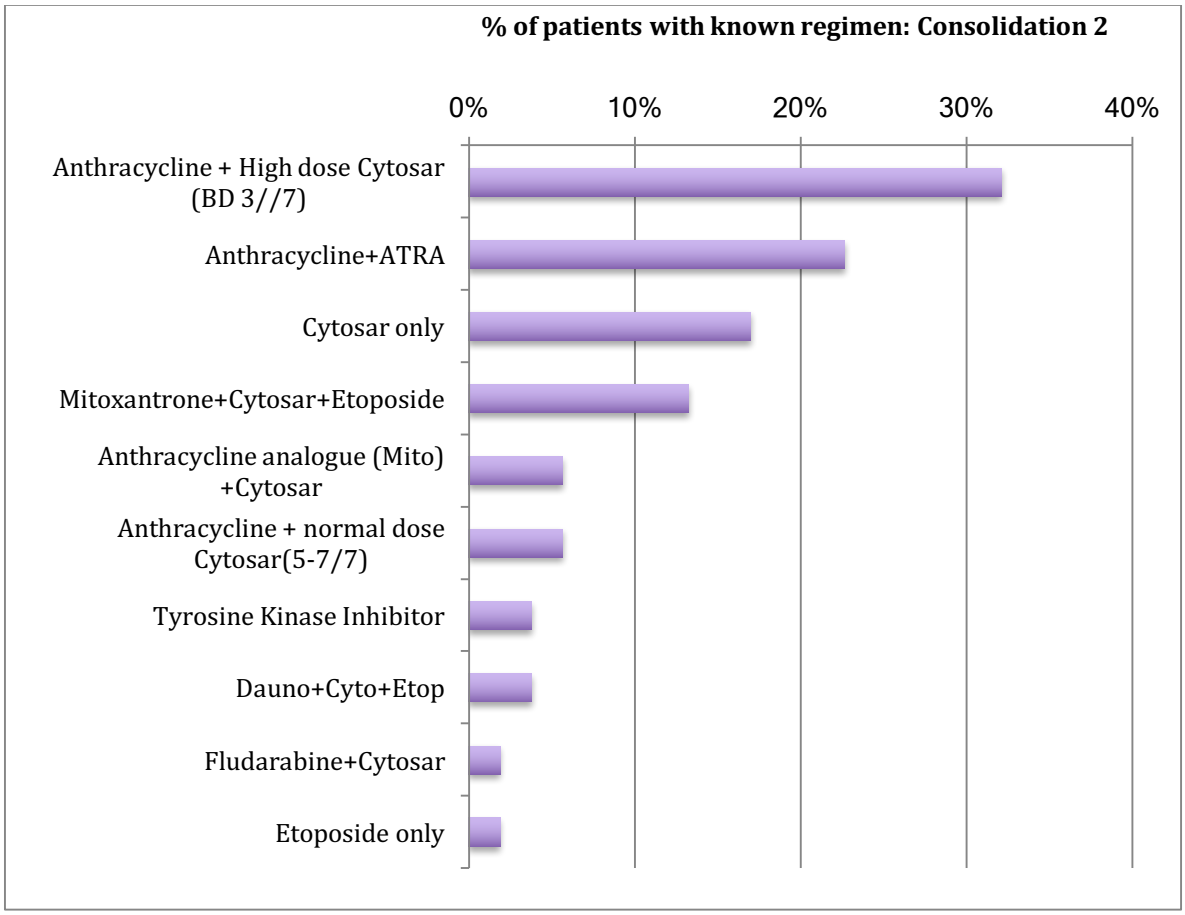


Figure 3.12: Chemotherapy regimens administered during consolidation cycle 2 in AML patients

Consolidation cycle 3

A further 65% of patients successfully treated with a second cycle of consolidation chemotherapy, proceeded to receive a third cycle of consolidation chemotherapy. The most common regimen administered for this cycle of chemotherapy was a combination of an anthracycline (daunorubicin) and high-dose cytosine arabinoside. This regimen was administered in 31.4% of patients. There were no significant differences in the chemotherapy regimens administered during the third cycle of consolidation between the HIV seronegative and the HIV seropositive patients. The frequency of regimens administered for the third cycle of consolidation chemotherapy are summarized in figure 3.13 below.

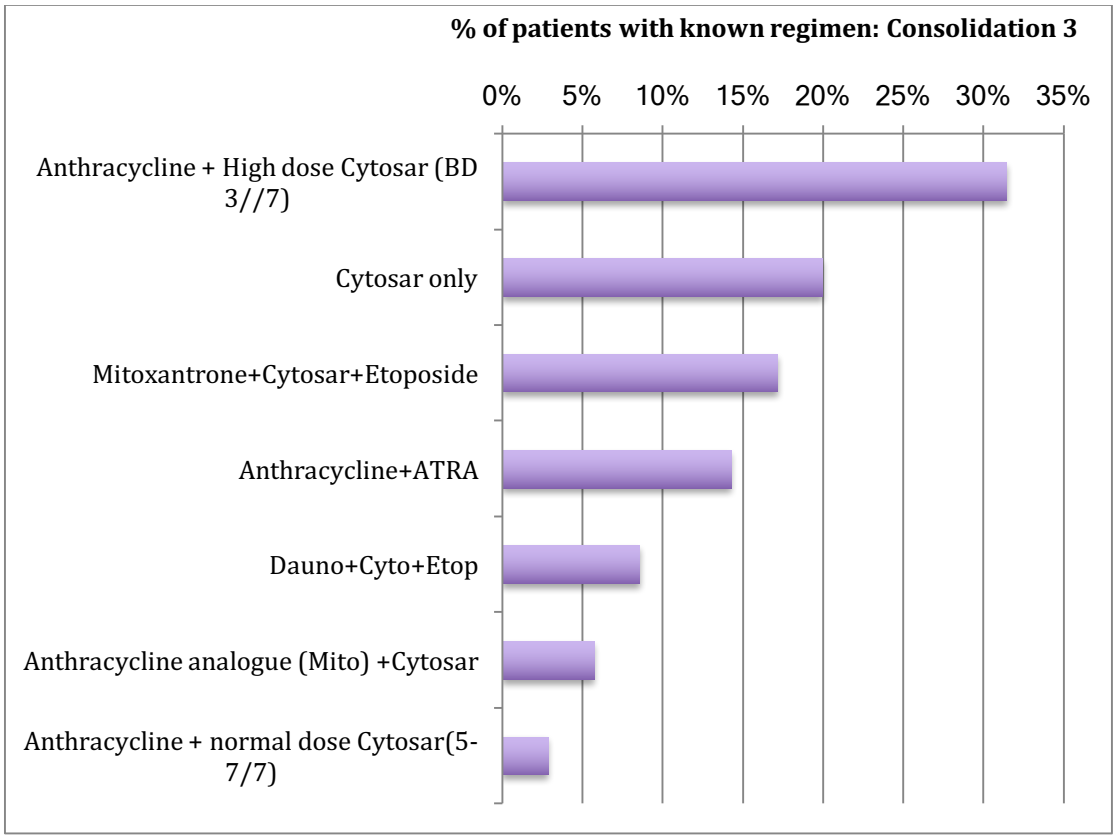


Figure 3.13: Chemotherapy regimens administered during consolidation cycle 3 in AML patients

Consolidation cycle 4

A total of 54% of patients reached consolidation cycle 4, during their initial chemotherapy course. The most common regimen administered for this cycle of chemotherapy was a combination of anthracycline (daunorubicin) and high dose cytosine arabinoside. This regimen was administered in 38% of patients who received consolidation cycle 4 chemotherapy. There were no significant differences in the chemotherapy regimens administered during this cycle of consolidation between the HIV seronegative and the HIV seropositive patients.

3.11. Maintenance chemotherapy

Approximately 9.9% (18/182) of the total study population received maintenance chemotherapy. All of these patients had AML M3 (acute promyelocytic leukaemia). The drug regimen administered as maintenance chemotherapy in all of these patients was a combination of all-transretinoic acid (ATRA), mercaptopurine and methotrexate. The administration of maintenance chemotherapy was similar between the HIV seronegative and the HIV seropositive patients.

3.12. Palliative chemotherapy

Among patients diagnosed with AML, 36/189 (19%) were treated with palliative chemotherapy. In this group of patients, 32/36 (89%) received oral thioguanine at a dose of 40 mg daily while 11% (4/36) of the patients received low dose intravenous cytosine arabinoside.

3.13. Outcomes

3.13.1. Remission

In this study, 60.7% of patients achieved remission after the first cycle of induction chemotherapy, the remaining 39.3% of patients failed to achieve remission after the first cycle of chemotherapy. In the subgroup analysis, among the patients who successfully achieved remission, 62.5% were HIV seronegative and 37.5% were HIV seropositive. This difference was close to, but did not achieve statistical significance, with a p-value of 0.054. Of the patients who achieved remission, 30% had confirmed relapse of their disease and later required a second course of induction chemotherapy. Following a second course of induction chemotherapy, only 24% of patients achieved a remission and 45% of these patients who achieved a remission suffered a second relapse during the course of their disease.

3.13.2. Patient status at follow up

A total of 76% (144/189) patients in this study demised with 132 patients having died from AML, while in 12 patients the cause of death was unknown. The number of patient who were alive was 5.8% (11/189). Among the patients who were alive, 10 patients were in remission while 1 patient was alive with confirmed disease relapse. In the subgroup analysis, the numbers of demised patients were similar between the HIV seronegative (75%) and HIV seropositive patients (81%). The proportion of patients who were alive in the HIV seronegative (25%) and the HIV seropositive (19%) groups were also similar. There were 18% (34/189) of patients who were lost to follow up. The status of disease at follow up is summarized in figure 3.14 below.

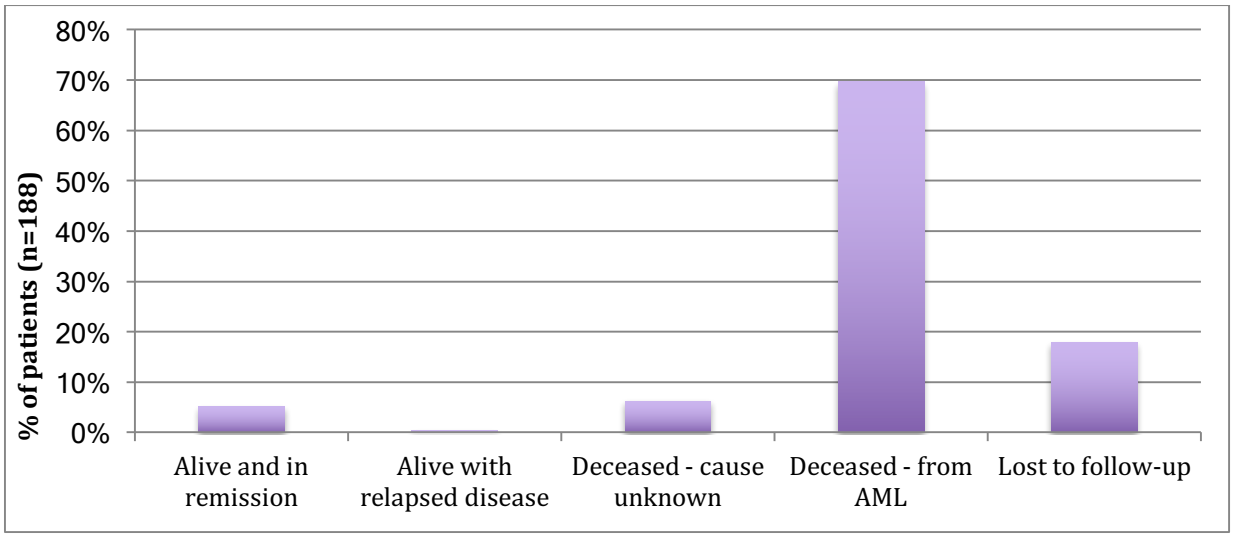


Figure 3.14: Disease status at follow up

3.13.3. Causes of death in patients with AML

Regarding the patients who demised, the most common cause of death was an infectious complication; this occurred in 81% of patients who demised. There was no difference in the occurrence of infection as a cause of death between the HIV seronegative and HIV seropositive patients with a p-value of 0.43. Haemorrhage was the cause of death in 15% of patients. There was no difference between the HIV seronegative and the HIV seropositive patients with a p-value of 0.30. Disseminated intravascular coagulopathy (in the absence of clinical evidence of infection) resulted in death in 8% of patients. There was no difference between HIV seronegative and HIV seropositive patients, with a p-value of 0.16. In some patients more than one event was documented as the cause of death, therefore the total percentages do not add up to 100%. The "other "category represents death events that were independent of the AML diagnosis. The prevalence of the different death events is summarized in figure 3.15 below.

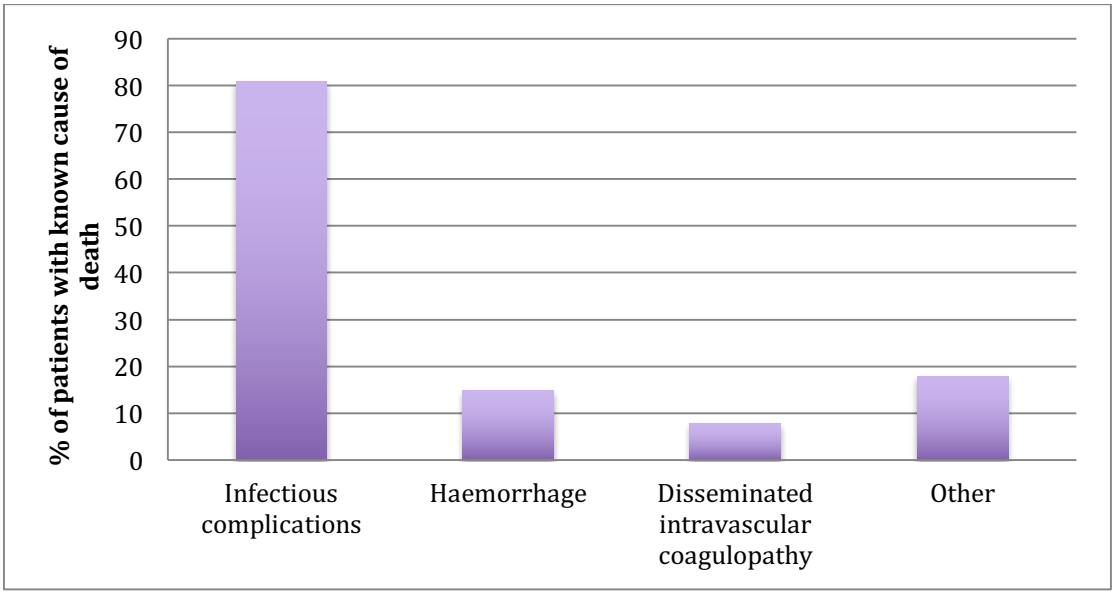
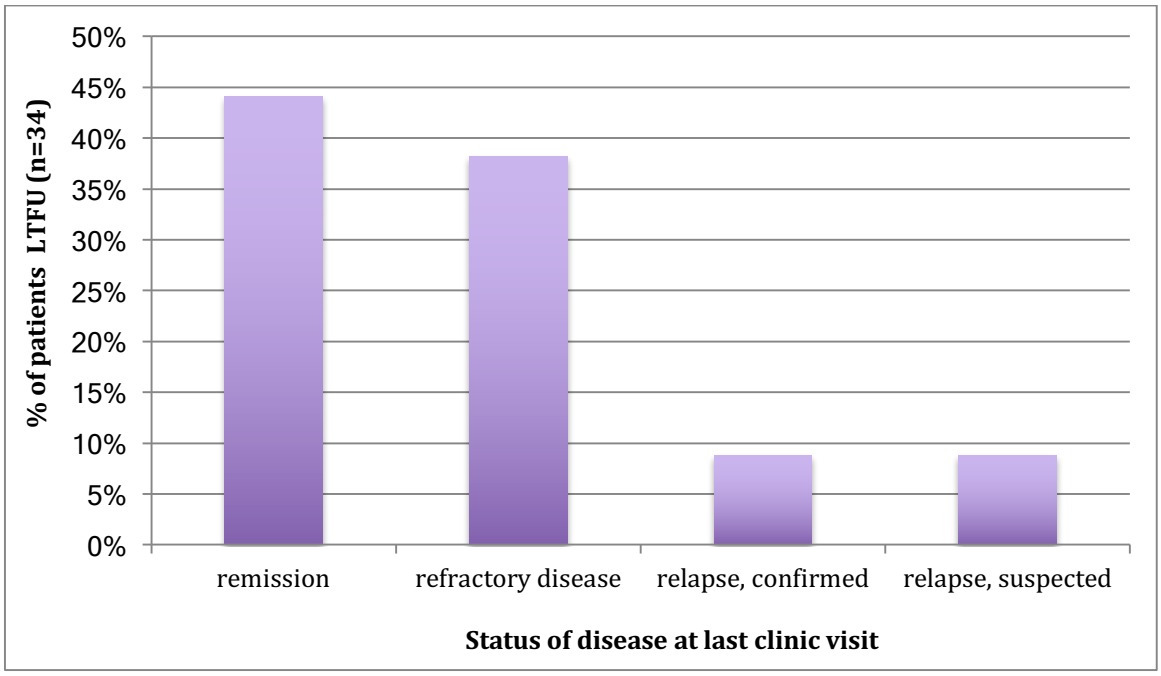


Figure 3.15: Causes of death in patients with AML

3.13.4. Lost to follow up

In this study, 34 patients were lost to follow up, with 30 patients being from the HIV seronegative AML group. The disease status of the lost to follow up patients, as documented at the last clinic visit was as follows: 44% of patients were in remission, 38% of patients had refractory disease, and a further 9% of patients had either confirmed or suspected disease relapse. The distribution of disease status in patients that were lost to follow up is shown in figure 3.16 below.



LTFU= lost to follow up

Figure 3.16: Patients lost to follow up by disease status at last clinic visit

3.14. Refractory disease

In this study, 38% (70/189) of the patients had disease that was refractory to chemotherapy. Among these patients, 59% (41/70) had never achieved remission at any point during the course of treatment due to primary refractory disease, while 41% of patients had secondary refractory disease. The patients with secondary refractory disease had achieved remission at some point during the course of treatment only to relapse later and have persistent disease despite subsequent cycles of chemotherapy. In the subgroup analysis of patients with refractory disease, 82.9% (58/70) were HIV seronegative, accounting for 36.7% of the HIV seronegative AML population. This is compared to 17.1% (12/70) patients who were HIV seropositive, accounting for 44.4% of the HIV seropositive study population. This difference in refractory disease between the HIV seronegative and the HIV seropositive patients was not statistically significant with a p-value of 0.39.

The proportion of primary to secondary refractory disease is represented n figure 3.17 below.

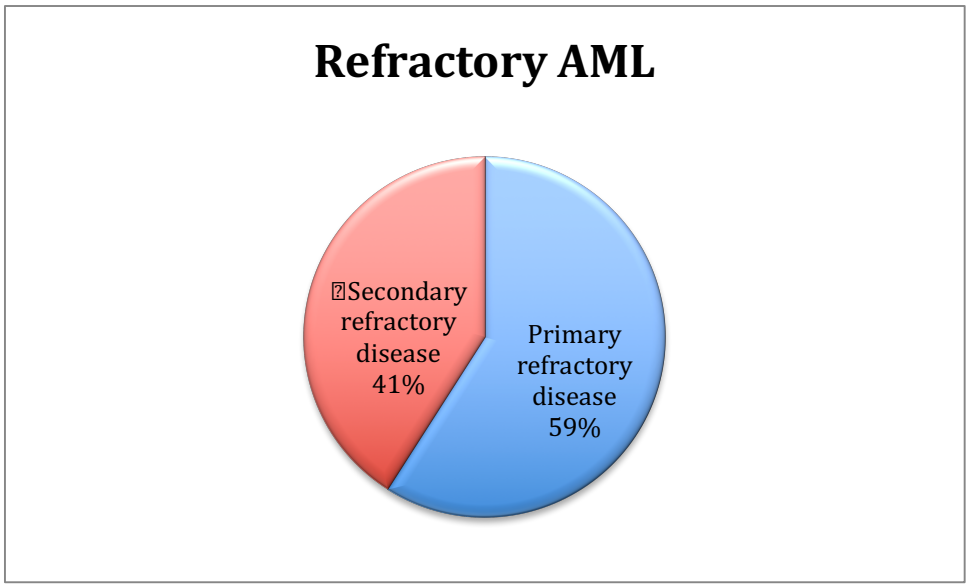


Figure 3.17: Frequency of primary versus secondary AML

3.15. Survival

The overall median survival was 0.4 years, (95% confidence interval: 0.2-0.7 years). There was no significant difference in survival between the HIV seronegative and HIV seropositive patients (p-value of 0.40). The survival curve is shown in figure 3.18 below.

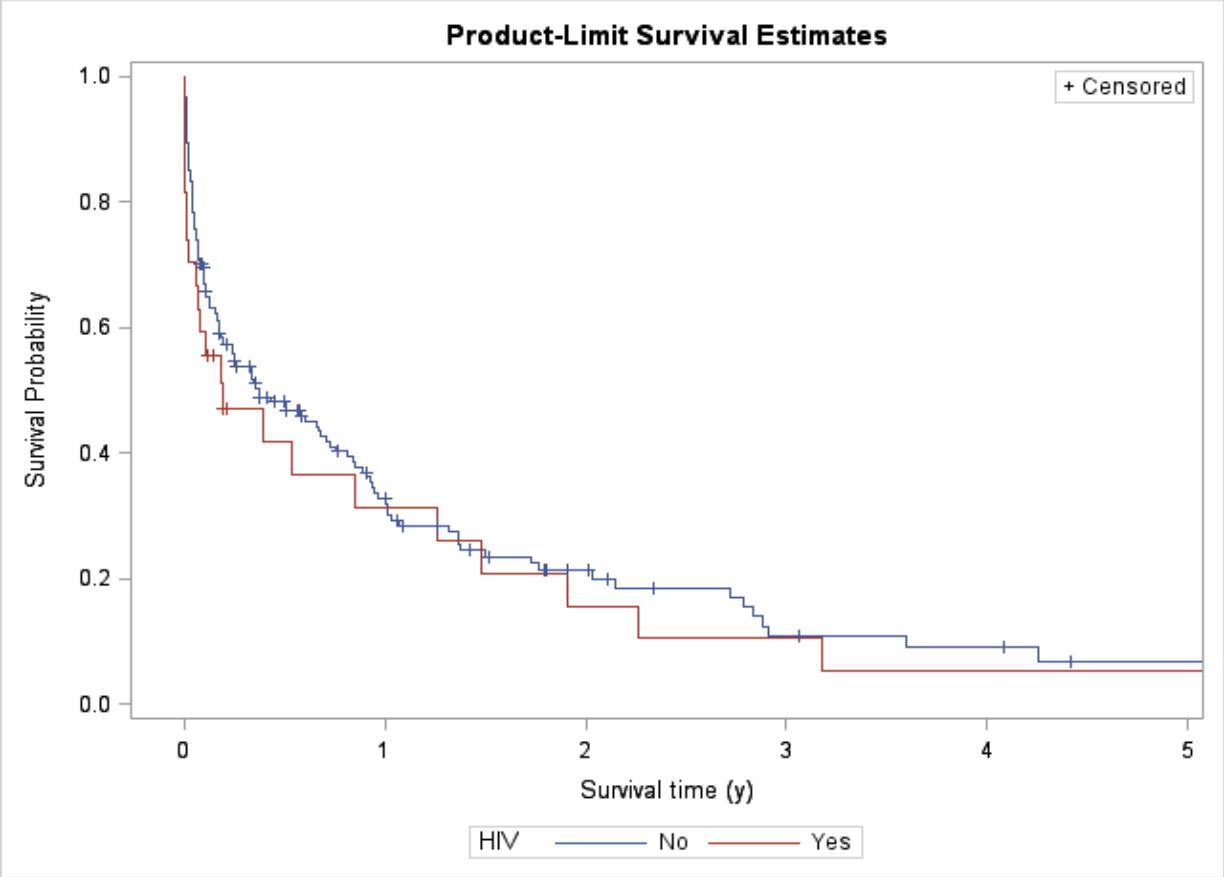


Figure 3.18: Survival of HIV seronegative and HIV seropositive patients with AML

The 1-year survival rate is 32.5% and by 5 years, the probability of survival drops to 6.5%. The survival estimates for 6 months, 1 year, 3 years and 5 years, together with their 95% confidence intervals, are presented in table 3.10 below.

Table 3.10: Annual survival rates of patients with AML

Time (years)	Survival (%)	95% CI for survival (%)
0.5	47.2%	39.7%-54.4%
1	32.5%	25.4%-39.9%
3	10.9%	5.8%-17.7%
5	6.5%	2.5%-13.2%

When the survival time is adjusted to exclude patients with early mortality within 0-3 months of being diagnosed with AML, the median survival time was 1.4 years (95% confidence interval: 0.9-1.9 years). There was no significant difference in survival between the HIV seronegative and HIV seropositive patients, with a p value of 0.98. The survival curve is shown in figure 3.19 below.

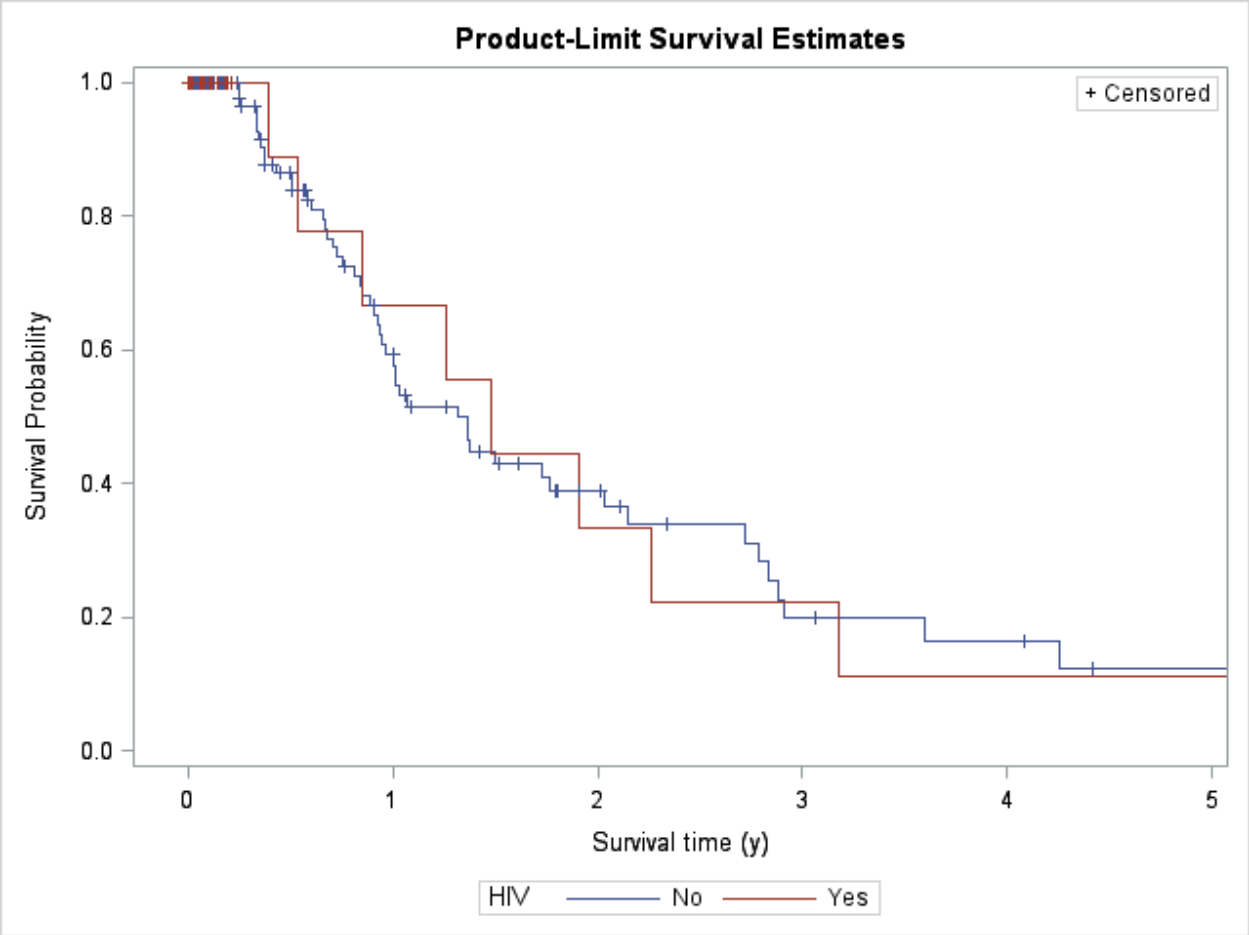


Figure 3.19: Survival of HIV seronegative and HIV seropositive patients with AML, excluding patients with early mortality (within 0-3 months of diagnosis)

The survival estimates for 6 months, 1 year, 3 years and 5 years, together with their 95% confidence intervals, are presented in table 3.11 below.

Table 3.11: Annual survival rates in patients with AML, excluding patients with early mortality (within 0-3 months of diagnosis)

Time (years)	Survival (%)	95% CI for survival (%)
0.5	86.7%	77.7%-86.7%
1	60.1%	48.6%-60.1%
3	20.3%	10.8%-20.3%
5	12.2%	4.6%-12.2%

3.16. Case reports

For the study period 01/01/1993-31/12/2004 there were 6 HIV seropositive patients with AML who were evaluated separately as a case series.

3.16.1. Patient 1

A 22-year-old male, employed as a petrol attendant, was initially admitted in June of 1993 with a 2-week history of symptoms of anaemia, significant loss of weight and night sweats. Clinical examination revealed the presence of shotty generalized lymphadenopathy, oral candidiasis and a mild hepatomegaly with no splenomegaly. The patient was also newly diagnosed with HIV with a CD_4 that represented 10% of the total lymphocyte count as well as a CD_4:CD_8 ratio of 0.77:1. The initial full blood count (FBC) showed a white cell count (WCC) of 0.53 $x10^9$/l, haemoglobin (Hb) of 6.6 g/dl and a platelet count of 25 $x10^9$/l. The bone marrow aspirate and trephine biopsy revealed the presence of a hypercellular marrow with approximately 75-80% myeloblasts. The myeloperoxidase reaction was negative. The patient was diagnosed with an undifferentiated acute myeloid leukaemia (AML MO).

The patient was treated with mitoxantrone. The response to chemotherapy however was poor and short lived with the patient developing cytopenias complicated by worsening nosocomial infections and haemorraghic varicella zoster. He survived for only one month following diagnosis with the date of death reported as July of 1993. He was never initiated on antiretroviral therapy.

3.16.2. Patient 2

A 37-year-old female who was known to be HIV seropositive, presented in May of 1994 with a 2-week history of nausea, vomiting and diarrhea, combined with loss of weight, night sweats and a dry cough. She was diagnosed with HIV eight months prior to this presentation (in September of 1993). Her risk factors for HIV transmission included previous transfusions for anaemia. She had never been treated with antiretroviral drugs. Clinical examination revealed the presence of oral candidiasis, shotty cervical and inguinal lymphadenopathy, gum hypertrophy and a 4 cm hepatomegaly and no splenomegaly. The initial FBC showed a high WCC of 24 $x10^9$/l with 60% myeloblasts demonstrated on the peripheral blood smear, haemoglobin of 5.9 g/dl and a platelet count of 13 $x10^9$/l. The bone marrow aspirate

and trephine biopsy showed extensive marrow infiltration by an acute leukaemia with approximately 89% of blasts. The myeloperoxidase reaction was positive in occasional mononuclear cells. The patient was diagnosed with AML (M1) with a normal XY karyotype.

The patient received an induction regimen that comprised of cytosine arabinoside. There was a poor response to chemotherapy, with a minimal decrease in the peripheral blast percentage from 60% to 44%. The patient demised 5 days later from progressive leukaemia.

3.16.3. Patient 3

A 38-year-old male was diagnosed with AML (M2) in June of 1994. He was also diagnosed with HIV during this admission. He received induction chemotherapy with mitoxantrone, successfully achieving remission. He was only given a single cycle of consolidation chemotherapy with mitoxantrone. He was never initiated on antiretroviral therapy. His follow up was erratic due to multiple periods of defaulting, which was mainly due to financial constraints and he was lost to follow up for a period of 5 months. The patient presented in May of 1995 with relapse. At relapse, he had fever, a productive cough, loss of weight, night sweats and symptoms of anaemia. His blood cultures were positive for a gram-negative bacillus. He was started on intravenous antibiotics and neutropenic measures. The FBC on this admission revealed a WCC of 3.8 $x10^9$/l, haemoglobin of 6 g/dl, a platelet count of 10 $x10^9$/l and peripheral blood blast percentage of 89%. The patient was given a second course of induction chemotherapy with mitoxantrone, but he failed to achieve a remission. A third cycle of induction chemotherapy was given with a combination of etoposide and cytosine arabinoside, which again failed to induce remission.

The patient was counselled regarding his prognosis but opted to have ongoing intravenous chemotherapy. Salvage chemotherapy in the form of cytosine arabinoside was given, however the patient's clinical condition continued to deteriorate. He had severe cytopenias requiring daily transfusion of blood products. He eventually succumbed to severe hospital acquired pneumonia in November of 1995, a total of 17 months after he was initially diagnosed with AML.

3.16.4. Patient 4

An 18-year-old female who initially presented to the surgical unit with a 3-day history of haematemesis, malaena stools, bleeding gums and epistaxis in February of 1995. On clinical examination, she was noted to be pale with significant axillary lymphadenopathy but no hepatosplenomegaly. The FBC on admission showed a WCC of 2.71 $x10^9$/l, haemoglobin of 9.2 g/dl and a platelet count of 434 $x10^9$/l The INR was 1.05. The bone marrow aspirate and trephine biopsy (BMAT) showed a hypercellular marrow with 1% blasts and 5% promyelocytes. The myeloperoxidase reaction was strongly positive. On cytogenetic assessment, a translocation (15; 17) was found and a diagnosis of acute promyelocytic leukaemia was made. During this admission she was also newly diagnosed with HIV. The baseline CD_4 count was 410 cells/ul.

The patient was started on all-transretinoic acid as a single agent and responded well to the treatment. The repeat BMAT performed in March of 1995 showed morphological remission. She was never treated with antiretroviral therapy. The patient was subsequently discharged to follow up. However, she was lost to follow up since March 1995.

3.16.5. Patient 5

A 30-year-old male presented in June of 2000 with a 1-month history of a sore throat, gum bleeding and symptoms of anaemia. On clinical assessment he was noted to have features of a purulent tonsillitis with associated pharyngitis. His FBC revealed a WCC 70 $x10^9$/l, haemoglobin of 6.1 g/dl and a platelet count of 33 $x10^9$/l. The peripheral smear had 90% abnormal promyelocytes. He was newly diagnosed with HIV and his CD_4 count was 554 cells/ul and HIV viral load was 37 300 copies/ml. A bone marrow aspirate and trephine revealed a marrow that was extensively infiltrated by 89% promyelocytes, morphologically in keeping with acute promyelocytic leukaemia, AML (M3). Fluorescent-in-situ hybridization (FISH) confirmed the presence of translocation (15; 17). The patient was given induction chemotherapy with a combination of daunorubicin, cytosine arabinoside and all-transretinoic acid (ATRA). A follow up bone marrow assessment confirmed morphological remission and FISH was negative for the PML-RARA gene. A total of 3 cycles of consolidation chemotherapy were given using the same chemotherapy combination as used during induction. The patient was initiated on cART by the infectious disease team.

The patient was well for a period of 3 months until he suffered a relapse of APL in January 2001. On this presentation he had a CD_4 count of 433 cells/ul and HIV viral load of 1285 copies/ml. He was given a new course of induction chemotherapy with mitoxantrone, cytosine arabinoside but he failed to achieve remission. An immediate course of reinduction was repeated with mitoxantrone and cytosine arabinoside, after which he successfully achieved remission. He went on to receive a further 2 cycles of consolidation chemotherapy. This period of remission was maintained for 15 months on maintenance oral therapy with mercaptopurine, methotrexate and ATRA.

In September of 2002, only 2 months after maintenance therapy was discontinued, 11% promyelocytes were detected on a full blood count during a routine follow up visit. He was treated for this relapse with a combination of daunorubicin, cytosine arabinoside and ATRA, followed by one cycle of consolidation with mitoxantrone and cytosine arabinoside. The patient remained well for 8 months.

In June of 2003, the patient was readmitted with a 3-day history of headache, fever and confusion. Clinically he was noted to have oral candidiasis, nuchal rigidity and pyrexia. The cerebrospinal fluid analysis was in keeping with a mixed meningitis with features of both bacterial and mycobacterial infection. On this admission his CD_4 count was 174 cells/ul. The patient was subsequently started on intravenous antibiotics and empiric anti-TB treatment. Unfortunately, his neurological status deteriorated when he developed a hemiplegia. Computerized tomography of the brain revealed marked basal meningeal enhancement with a focal area of possible cerebritis in the left posterior aspect of the temporal lobe. A repeat analysis of the cerebrospinal fluid revealed a marked lymphocytosis of 634 cells/ul and a protein level of 3.2 g/l. The patient showed a poor response to treatment and subsequently succumbed to progression of the disease and associated complications in July of 2003, a month after his second relapse and a total of 38 months from the initial diagnosis of AML.

3.16.6. Patient 6

A 39-year-old female who was known to be HIV seropositive, and on antiretroviral therapy receiving a combination of zidovudine, lamivudine and nevirapine. She was previously diagnosed and treated in 1997 for biopsy proven Kaposi sarcoma of the lung. Etoposide was part of the chemotherapeutic regimen. She presented in February of 2000 with symptoms of anaemia and bleeding (epistaxis, gum bleeding and easy bruising). On examination, she was pale and had oral candidiasis. She was also noted to have a pelvic mass that was thought to be of uterine origin on clinical examination. The initial FBC revealed a WCC of 3.4 $x10^9$/l, haemoglobin of 8.9 g/dl and a platelet count of 28 $x10^9$/l, with 14% blasts noted on the peripheral blood. Her CD_4 count at presentation was 193 cells/ul and her HIV viral load was >750 000 copies/ml. The bone marrow aspirate and trephine biopsy revealed extensive marrow infiltration by an acute myeloid leukaemia fitting best with acute promyelocytic leukaemia. The FISH analysis was positive for the PML/RARA fusion gene, with the flow cytometry also in keeping with AML (M3).

The patient was given all-transretinoic acid (ATRA) and blood products for the worsening cytopenias. A trans-abdominal ultrasound revealed a large multi-loculated complex fluid collection with enhancement of the walls of the rectum and bladder in keeping with a retroperitoneal haematoma. This was initially treated conservatively with colpocentesis, however the haematoma continued to expand causing rectal and bladder compression necessitating evacuation by laparotomy. Unfortunately, the patient had a complicated post-operative course with abdominal sepsis and septic shock. Despite inotropic support and appropriate intravenous antibiotics, the patient demised in March 2000, a month after her initial diagnosis of AML.

A summary of all the HIV seropositive patients with AML who were included as a case series can be found in table 3.12 below, and a summary of all the HIV seropositive patients with AML who were diagnosed and treated at Chris Hani Baragwanath hospital from 01/01/1993-31/12/2014 is included as table 3.13 below.

Table 3.12. Case reports: HIV seropositive patients with AML from 1993-2004

Patient Age/Gender (M/F)	Duration between HIV and AML diagnosis (mths)	Clinical presentation	Clinical examination	Laboratory results WCC $x10^9$ Hb g/dl PLTS $x10^9$ Blast %	FAB subtype/ Marrow features	CD4 count /HIV viral Load	Treatment /cART	Outcome/survival (days/mths)	Cause of death
Patient 1 22, M	0	Symptoms of anaemia, weight loss, night sweats	Shotty generalized LAD, mild hepatomegaly	0.53 6.6 25 N/A	MO Hypercellular, 75-80% blasts	N/A	Mitoxantrone Not given cART	Demised Survival: 1 mth	Severe cytopenias. Neutropenic sepsis Haemorrhagic VZV
Patient 2 37, F	8	Nausea and vomiting, weight loss, night sweats, dry cough	Shotty cervical and inguinal LAD, gum hypertrophy, 4cm hepatomegaly	24 5.9 13 60%	M1 Extensive leukaemic infiltration	N/A	Cytosine arabinoside Not given cART	Demised Survival: 5 days	Progressive leukaemia
Patient 3 38, M	0	Symptoms of anaemia, fever, cough, weight loss, night sweats	Pyrexial, oral candidiasis	3.8 6 10 89%	M2	N/A	Induction 1 and consolidation1: mitoxantrone Induction 2: mitoxantrone Induction 3: etoposide+cytosar Palliative: cytosar Not given cART	Demised Survival: 17 mths	Nosocomial pneumonia
Patient 4 18, F	0	Bleeding: haematemesis, epistaxis bleeding gums	Axillary LAD	2.7 9.2 434 N/A	M3 Hypercellular with abnormal promyelocytes	410 N/A	ATRA Not given cART	Lost to follow up, in remission. Survival: 2mths	-

Patient Age/Gender (M/F)	Duration between HIV and AML diagnosis (mths)	Clinical presentation	Clinical examination	Laboratory results WCC $x10^9$ Hb g/dl PLTS $x10^9$ Blast %	FAB subtype/ Marrow features	CD4 count /HIV viral Load	Treatment /cART	Outcome/survival (days/mths)	Cause of death
Patient 5 30, M	0	Sore throat gum bleeding, symptoms of anaemia	Purulent tonsillitis	70 6.1 33 90%	M3 Extensive infiltrate of 89% promyelocytes FISH t(15; 17)	554 37300	Induction 1+ 3 cycles of consolidation: daunorubicin+cytosar+ATRA New course: reinduction mitoxantrone+cytosar Maintenance with MMA New course: reinduction daunorubicin+cytosar+ATRA Consolidation mitoxantrone and cytosar. cART	Achieved remission, relapsed after 3 mths Achieved remission, relapsed after 15 mths Achieved remission, relapsed after 8 mths Demised Survival: 38 mths	Complicated Mixed meningitis (bacterial and tuberculous)
Patient 6 39, F	36	Treated previously for Kaposi's Sarcoma with etoposide containing regimen. Symptoms of bleeding: epistaxis, gum bleeding	Oral candida Pelvic mass	3.4 8.9 28 14%	M3 Abundant promyelocytes FISH: t (15, 17)	193 >750 000	ATRA cART: zidovudine+lamivudine+ nevirapine	Demised Survival: 1mth	Surgical complications: intra-abdominal sepsis

cART= combination antiretroviral therapy, LAD= lymphadenopathy, N/A= not available, VZV= varicella zoster virus, Cytosar=cytosine arabinoside, mth(s)= month(s), ATRA= all-transretinoic acid, MMA: mercaptopurine+methotrexate+ATRA,FISH=fluorescent-in-situ hybridizaton

Table 3.13. Summary of HIV seropositive patients with AML

Parameter	HIV seropositive patients 1993-2014 N=33
Age (mean)	37 years (range: 18-74 years)
Gender	
Male	14 (42.4%)
Female	19 (57.6%)
Male: female ratio	1:1.4
Symptoms	
Anaemia	29 (93.5%)
Bleeding	16 (53.5%)
Bleeding gums	12 (75%)
Skin	6 (37.5%)
Epistaxis	7 (43.8%)
Malaena	2 (12.5%)
Menorrhagia	4 (25%)
Haemoptysis	1 (6.3%)
Haematuria	1 (6.3%)
Haematemesis	1 (6.3%)
Infections	9 (27.3%)
Respiratory	8 (88.9%)
Gastrointestinal	1 (11.1%)
Central nervous system	1 (11.1%)
Constitutional symptoms	
Weight loss	19 (57.6%)
Fever	21 (63.6%)
Night sweats	18 (54.5%)
Bone pain	5 (16.7%)
Clinical signs	
Pallor	31 (93.9%)
Lymphadenopathy	12 (36.4%)
Signs of bleeding	
Ecchymosis	11 (33.3%)
Petechiae	7 (21.2%)
Purpura	5 (15.2%)
Evidence of infection	
Respiratory	11 (91.7%)
Genitourinary	1 (8.3%)
Central nervous system	1 (9.0%)
Tuberculosis	7 (22.6%)
Organomegaly	
Hepatomegaly	13 (39.4%)
Splenomegaly	6 (18.2%)
Abdominal mass	2 (6.1%)
Extramedullary	
Gum hypertrophy	3 (9.1%)
Myeloid sarcoma	3 (9.1%)
Skin	2 (6.1%)

Parameter	HIV seropositive patients 1993-2014 N=33
De novo versus secondary aetiology	
De novo (primary)	29 (87.9%)
Secondary	4 (12.1%)
Myelodysplasia	2 (6.1%)
Chemotherapy related	2 (6.1%)
FAB histological subtype	
M0	2 (7.7%)
M1	1 (3.8%)
M2	11 (42.3%)
M3	10 (38.5%)
M4	2 (7.7%)
M5/M6/M7	0
Unknown	7
CD_4 cells/ul (mean)	350 (range: 29-1379)
HIV viral load copies/ml (mean)	185 265 (range: 0-750 000)
Management	
Received chemotherapy	
Yes	26 (78.8%)
No	7 (21.2%)
Maintenance chemotherapy	4 (12.1%)
Palliative chemotherapy	3 (9.1%)
Combination antiretroviral therapy	20 (60.6%)
Outcome	
Alive	0
Demised	27 (81.8%)
Lost to follow up	6 (18.2%)
Survival (median in years)	0.3 years.

FAB = French American British classification.

CHAPTER 4: DISCUSSION

4.1 Demographics of AML

Acute myeloid leukaemia is a haematological malignancy that occurs mainly in the elderly. Due to its demographics, it occurs less frequently in the setting of HIV infection. In this study the records of 189 patients with AML were reviewed over a ten-year period from 01/01/2005 to 31/12/2014, with an additional 6 HIV seropositive patients with AML reviewed for the period from 01/01/1993 to 31/12/2004. An overwhelming majority of patients in the study population were of Black African decent, which represents the patient demographic of Chris Hani Baragwanath Academic Hospital. There was a predominance of men compared to women in this study, with a male: female ratio of 1.1:1, which is in keeping with the known male predominance in AML (1). Interestingly, the HIV seropositive group demonstrated a female predominance of 59% with a male: female ratio of 1:1.5. This reflects the demographics of HIV in Sub-Saharan Africa, which carries 70.8% of the global burden of HIV, and 57% of this HIV seropositive population are females (39).

The median age at presentation in this study population was 45 years (IQR 18-88 years). The age at presentation in our study is lower than what has been reported in the literature i.e. the seventh decade of life with an average of 67-70 years (1, 5). A similar younger age of AML of 41 years was also found in a study carried out by Marshall et al., at the National Health Laboratory Services (NHLS), at the University of the Witwatersrand. In the HIV seropositive patients with AML, the age at presentation was statistically significantly lower, at a median of 38 years (p-value of 0.027). This is largely attributable to the general trends of HIV seropositive patients in the South African setting, where the highest incidence of HIV as a cause of disability is seen in patients between the ages of 30-44 years (39). Haematological malignancies occurring in HIV seropositive patients have been documented in younger age groups although these have been mainly of the lymphoid lineage (29, 30). Furthermore, the demographics of the HIV seropositive population in South Africa are different from that encountered in Europe and the Americas where the HIV seropositive population is comprised mainly of older homosexual male patients. This may account for the HIV seropositive AML patients encountered in our study being predominantly

younger females and where the predominant risk factor for HIV infection is typically heterosexual contact.

4.2. Aetiopathogenesis of AML

In the aetiopathogenesis of AML in this study, the most common risk factor identified for AML was occupational exposure found in 5% of patients. Patients who were previously treated for a malignancy with exposure to cytotoxic drugs comprised 3.7% of the patients in our study. Although de novo AML predominated, secondary AML was documented in 31% of the total study population. The HIV seronegative AML patients were statistically significantly more likely to develop secondary AML than their HIV seropositive counterparts (p-value 0.016). The most common aetiology of secondary AML in this study population was pre-existing myelodysplasia with leukaemic transformation, being present in 51% of patients with secondary AML. There was no difference in the incidence of myelodysplasia between the HIV seronegative and HIV seropositive patients. The evolution of secondary AML from myelodysplastic syndromes is well documented and occurs as a progression of disease in up to a third of patients (44, 45). A possible reason that has been cited for AML occurring in the setting of HIV infection includes the interaction of the human immunodeficiency virus with the marrow micro-environment which may affect cellular proliferation and potentially lead to myelodysplasia and malignant transformation (36). The features of HIV related myelodysplasia were found in only one patient in this study and remains an extremely rare cause of AML in this setting.

Myeloproliferative disorders, in particular chronic myeloid leukaemia (CML) undergoing blastic transformation was responsible for 39% of secondary AML. The incidence of patients who develop AML following a course of chemotherapy, with either an alkylating agent or topoisomerase II inhibitor, has been cited to be as high as 10-15% in some studies (1, 44). In this study, however, the incidence of therapy related AML was very low, at 3.7% of the overall study population.

4.3. Clinical presentation of patients with AML

Patients are typically referred to us with AML following the detection of blasts on the peripheral blood smear. The clinical presentation in these patients is usually with symptoms of bone marrow failure/infiltration. This is the most commonly described clinical presentation of AML in the literature (1, 40). The prevalence of symptoms of anaemia, bleeding and infections was similar for the HIV seronegative and HIV seropositive groups. Symptoms of anaemia are the most common presenting feature, present in 92 % of the patients; this correlates with pallor/anaemia, being the most common clinical sign in 94% of the patients.

With regard to infections as a presenting feature, the respiratory tract was the most common site of infection (68%). There was a correlation between reported symptoms and the clinical evidence of infections across the organ systems with the exception of central nervous system infections which were more likely to be detected clinically due in part to the inability of the patient to accurately report these symptoms. Although there was no difference in the incidence of acute infections between the two study populations, the proportion of patients with tuberculosis was higher in the HIV seropositive patients with AML, compared to their HIV seronegative counterparts. This difference was statistically significant with a p-value of 0.0018. In the 16 patients with tuberculosis, pulmonary TB was the most common site of infection, while in 4 patients the diagnosis of tuberculosis was made on the presence of tuberculous bacilli on a bone marrow specimen submitted for the purpose of confirmation of the AML diagnosis. The symbiotic relationship between HIV and TB is well documented and tuberculosis remains the most common cause of mortality and morbidity among the HIV seropositive population of Southern Africa (39).

4.4. Extramedullary disease

It has been proposed that extramedullary disease in AML may occur more commonly in patients who are HIV seropositive, similar to extranodal disease occurring more commonly in HIV infected patients with lymphoproliferative malignancies (36, 40). Although the incidence of extramedullary disease was higher in this study at 21% of the overall population than found in other studies in the literature, the incidence of

extramedullary disease was no higher in the HIV seropositive patients compared to the HIV seronegative patients with AML.

Myeloid sarcoma in this study population occurred in 13/189 (6.9%) of the patients. Among the patients presenting with a myeloid sarcoma, only 3 were HIV seropositive. The presentation of a myeloid sarcoma is rare in AML; its incidence in AML ranges between 1-9.1% (1, 46). In view of the atypical and aggressive course that haematological malignancies tend to follow in HIV infection, it has been proposed that myeloid sarcomas may occur with a higher incidence among HIV seropositive patients with AML (36). This however, has not been conclusively shown in published studies. In our study, we were unable to show any statistically significant difference in the incidence of myeloid sarcomas between the HIV seropositive and seronegative patients.

Involvement of the reticuloendothelial system with the presence of lymphadenopathy, hepatomegaly and splenomegaly may be a clinical feature of uncomplicated HIV infection, thus it may be expected that HIV seropositive patients with AML may have a higher incidence of these clinical signs at presentation. This, however, was not a significant finding in this study. Generally these features are less commonly encountered in the setting of AML, than in the lymphoproliferative disorders.

4.5. Clinical presentation of HIV seropositive patients with AML

This study contains the largest case series of HIV seropositive patients with AML seen and treated at a single center. A total of 33 HIV seropositive patients with AML were evaluated at the Clinical Haematology unit at Chris Hani Baragwanath Academic hospital. In the majority of patients in this study (60%), the diagnosis of HIV was made simultaneously with the AML diagnosis, with these patients being antiretroviral treatment naïve. This differs from international data where most patients reported are older with an antecedent diagnosis of HIV, and are on antiretroviral therapy prior to the diagnosis of AML. Only a small number of HIV seropositive AML patients presented with clinically advanced HIV disease or AIDS, with 3 HIV seropositive patients having WHO clinical stage 4 disease, based on the presence of

extra-pulmonary tuberculosis. Indeed, infection with tuberculosis was the most evident complication of HIV infection that was noted in this study. Pulmonary and extra-pulmonary tuberculosis occurred with a higher incidence in the HIV seropositive patients, this finding was statistically significant with a p-value =0.0018.

The mean CD_4 count among HIV seropositive AML patients was 353 cells/ul (range 29-1379); predictably the CD_4 counts were lower among patients not on cART. Nevertheless, the CD_4 counts appear higher in our patients with AML than in a general HIV seropositive population and may be overestimated due to the leukaemic nature of the disease manifesting with higher leucocyte counts at presentation. The infection of haemopoietic progenitor cells by the human immunodeficiency virus, as a cause of leukaemogenesis in AML has never been proven, however it has been postulated that the degree of immunosuppression and depletion of T-lymphocytes caused by HIV significantly reduces immune surveillance and thus the clearance of leukaemic cells. This may be a risk factor not only for developing AML but may result in poorer outcomes in patients who are infected with HIV and particularly those with lower CD_4 counts < 200 cells/ul (40).

Eleven of the 12 the patients that had a preceding diagnosis of HIV infection were already on cART prior to developing AML, with a mean CD_4 count of 310 cells/ul (range 156-505) and a mean duration of therapy of 24 months (range 0.7-84 months). Only one patient required a change in the antiretroviral therapy regimen due to an adverse drug reaction. In the case series of 6 patients, 4 out of 6 patients were not on cART due to the limited availability of antiretroviral therapy in South Africa prior to the launching of the national treatment programme in 2004 (47).

4.6. Laboratory results

In the HIV seropositive patient the presence of cytopenias is not infrequent. The aetiology of the cytopenias is often multifactorial, with anaemia of chronic disease, presence of opportunistic infections, nutritional factors as well as adverse drug effects being possible risk factors. Regarding the full blood count, the HIV seropositive

patients with AML had lower overall leucocyte, haemoglobin and platelet counts when compared to their HIV seronegative AML counterparts. Although the findings were not statistically significant for any of these parameters, it is accepted that HIV seropositive patients have a higher incidence of cytopenias in the setting of AML. When present however, cytopenias may be assumed to be due to other causes and this may delay the investigation and subsequent diagnosis of AML in patients who are HIV infected (36).

Serum albumin was statistically significantly lower in the HIV seropositive (mean 31.4 g/l) compared to the HIV seronegative AML patients (mean 35.4 g/dl), p-value of 0.0064. Albumin is a well-known negative acute phase reactant, it tends to be low in the setting of chronic inflammation, protein and energy malnutrition and malabsorptive states, all of which can occur in the setting of HIV infection. With regards to haematinic levels, the HIV seronegative patients had higher ferritin levels compared to the HIV seropositive patients, this difference was significant with a p-value of 0.034. The elevated ferritin levels may reflect an acute inflammatory response to infections or the leukaemic process itself. The ferritin levels may therefore not be an accurate measure of iron stores in these patients. Further subgroup analysis of biochemistry parameters revealed that the HIV seropositive patients with AML had lower serum sodium and gamma glutamyl transferase levels compared to the HIV seronegative patients, the reason for these findings are unclear and may need to be explored in future studies.

4.7. Marrow elements

The presence of cellular dysplasia during histological evaluation of the bone marrow aspirate and trephine samples was reported in 30% of HIV seronegative AML samples and 26% of HIV seropositive AML samples. In the HIV seropositive group only one sample was noted to have HIV related myelodysplastic changes. This represents less than 4% of the total HIV seropositive study group. These findings confirm the notion that despite HIV related marrow dysplasia being an important cause of HIV related cytopenias, the progression of this dysplasia to overt leukaemia is still exceptionally rare (36).

4.8. Histological subtypes and cytogenetics

Overall, the most common histological subtype was AML M2 (myeloblastic with granulocytic maturation), being present in 25% of the study population. Indeed, AML M2 was more common in the HIV seropositive group, being present in 48% of these AML patients. The predominance of AML M2 in HIV seropositive patients has also been demonstrated in other studies (36, 40). Acute promyelocytic leukaemia – AML M3 was the next most common subtype representing 22% of the population. The high incidence of AML M3 is however, a unique finding in our study and may reflect the younger age at presentation of AML M3 patients with a mean age of 32 years (range: 16-57 years), coinciding with the younger age at presentation of HIV seropositive patients with a mean age of 39 years (range: 21-74 years).

With regard to cytogenetics, the most common favourable cytogenetic abnormality was translocation (15; 17), found in 96% of the patients with AML M3 subtype. There was no difference between HIV seropositive and HIV seronegative patients in this regard. The HIV seropositive patients with the AML M2 subtype in our study were more likely to express the favourable translocation (8; 21) cytogenetic abnormality when compared to the HIV seronegative AML M2 patients. This difference was statistically significant with a p-value of 0.0091. The inversion (16) cytogenetic abnormality, which is also viewed as a favourable cytogenetic abnormality was rare in this study population, with an overall prevalence of 2.4%.

Unfavourable cytogenetics occurred at a lower incidence in our study population. The most common unfavourable cytogenetic abnormality found in this study was translocation (9; 22), which occurred in 6.6% of the patients with presumed blastic transformation from chronic myeloid leukaemia to acute myeloid leukaemia. A complex karyotype, which is defined as the presence of at least 3 chromosomal abnormalities within a malignant cell (2), was found in 5 patients (3%). Only one of these patients was HIV seropositive. The chromosomal deletions, 7q- and 5q- were also infrequent, with a frequency of 6% and 2.4%, respectively. Abnormalities in chromosome 7 tend to be associated with myelodyplasia as well as chemotherapy

related AML. In our study, only one patient with previous exposure to cytotoxic agents displayed this abnormality. The presence of unfavourable cytogenetic abnormalities were no different in the HIV seronegative compared to the HIV seropositive group of AML patients. The low levels of unfavourable cytogenetics could again be due to the younger age of the patients in our study. The highest risk for unfavourable cytogenetic abnormalities in AML includes advanced age; therapy related AML as well as AML that evolves from a preceeding myeloproliferative neoplasm (11, 22).

4.9. Treatment and therapeutic response

In the treatment of AML, the chemotherapy regimen for remission induction has remained essentially unchanged over the past few decades with the combination of an anthracycline and cytosine arabinoside forming the backbone of treatment and serving as the standard of care for initial therapy (1, 2). In our study, 82% of the patients treated for AML received the standard induction chemotherapy. The majority of these patients (60%) received the standard "3+7" regimen. There were a lower proportion of HIV seropositive patients with AML (35%), who received the standard seven days of cytosine arabinoside when compared to the HIV seronegative patients with AML (65%). This difference between the two study populations was statistically significant with a p-value of 0.0015. The HIV seropositive AML patients in this study were more likely to receive a reduced duration of five days of cytosine arabinoside. This was largely due to more severe cytopenias and the greater potential for neutropenic complications.

In all of the patients treated, 61% achieved a remission after the first cycle of chemotherapy, while 39% of the patients who received the first cycle of induction chemotherapy failed to achieve remission. In the subgroup analysis of patients who failed induction chemotherapy, 62.5% were HIV seronegative and 37.5% were HIV seropositive. This difference was not statistically significant with a p-value of 0.054. This finding supports the fact that HIV seropositive patients treated intensively for AML are not more likely to fail induction chemotherapy and can therefore achieve similar remission rates as their HIV seronegative counterparts (29, 36, 40).

In patients who required further cycles of induction chemotherapy due to either relapsed or refractory disease, the most commonly administered regimen for re-induction cycles 1 to 3 was a combination of mitoxantrone, etoposide and cytosine arabinoside. Patients with refractory disease, who went on to require reinduction chemotherapy beyond cycle 3, were given a combination of fludarabine and cytosine arabinoside, which is a conventional recommended regimen for salvage therapy. One patient with primary refractory disease was given 2 courses of azacitidine; this drug has been shown to improve survival in patients who are unlikely to tolerate conventional salvage therapies, particularly in the elderly patient (2, 3).

With regard to consolidation chemotherapy, the most common regimen that was administered to patients who had achieved remission was a combination of daunorubicin with high dose cytosine arabinoside given over 1-2 hours, every 12 hours over three days (a total of 6 doses). This regimen was given through consolidation cycles 1 to 4 in the first chemotherapy course. Patients with acute promyelocytic leukaemia (AML M3) were given consolidation chemotherapy with a combination of daunorubicin and all-transretinoic acid, which is the standard of care.

Maintenance chemotherapy was only given to patients with the acute promyelocytic subtype of AML; all of these patients received a combination of mercaptopurine, all-transretinoic acid and methotrexate for a period of at least 2 years. A total of 36 patients in this study were treated conservatively and given palliative chemotherapy due to a poor clinical condition. The majority of these patients were HIV seronegative, with advanced age, a poor performance status as well as severe cytopenias. Regarding patients who received palliative chemotherapy, oral thioguanine was most commonly administered in 89% of these patients, while 11% of the patients received low dose cytosine arabinoside as a single agent.

In the setting of AML, palliative chemotherapy is generally given to control leukemic blast counts, when achieving a sustained remission with conventional intensive chemotherapy is not feasible. This is common practice for AML patients who are more elderly, have multiple co-morbid diseases or are clinically unfit (1). Although HIV seropositive patients are more likely to have disease processes that may render

them unsuitable candidates for intensive chemotherapy programmes, they have not been shown in the published literature or in our study to be more likely to receive palliative chemotherapy than their HIV seronegative counterparts.

4.10. Outcomes

With regard to disease outcome in our study, 76% of patients demised, 18% of patients were lost to follow up, and 6% were alive at the time of the study. Of the 11 patients that were alive at follow up, 10/11 patients were in remission and 1 patient had confirmed relapsed disease. A total of 34 patients in our study were lost to follow up, with 44% of these patients being in remission, while 38% of patients had refractory disease at their last clinic visit and may have possibly demised at home or at another health care facility. Of the patients that demised, and where the cause of death was known, the most common cause of death was neutropenic sepsis in both the HIV seronegative and seropositive patients. Neutropenic complications occurring as either progression of leukaemic disease or as a consequence of chemotherapy, is universally the most common cause of death among patients with AML (4).

4.11. Chemotherapy resistant AML

Refractory AML was present in 38% of the overall population evaluated in this study. Forty-one out of 70 patients (59%) had primary refractory disease, having never achieved remission at any point in time during their treatment. The remaining 29 patients (41%) with secondary refractory disease, who had previously achieved remission, and then subsequently relapsed, failed to respond to further cycles of chemotherapy. The incidence of refractory disease may reflect the incidence of secondary AML in our study. As previously stated, 31 % of patients in this study had secondary AML, the commonest aetiology of which was preceding myelodysplasia with leukaemic transformation. Myelodysplasia and therapy-related AML are more likely to be resistant to conventional chemotherapy than de novo AML (1). However, the majority of the patients with refractory disease in our study had de novo AML. This suggests that there may be molecular or genetic peculiarities in our AML population that contribute to chemo-resistance and which have not yet been explained

or properly clarified. The presence of an adverse cytogenetic abnormality and molecular aberrations are important predictors of chemotherapy resistant disease and adverse overall outcome in AML (1, 24, 48). Considering the predominance of favourable cytogenetic profiles detected in this study population, particularly in the HIV seropositive AML group, the incidence of chemotherapy resistant disease in this study population is higher than anticipated and further studies are needed to explain and assess the molecular and cytogenetic properties of AML, in the South African context.

In the subgroup analysis, patients with refractory disease comprised 38% of the HIV seronegative patients with AML and 46% of the HIV seropositive patients with AML, respectively. Although patients with refractory disease comprised a higher proportion of the HIV seropositive group, the difference was not statistically significant, with a p-value of 0.39. HIV infection alone has not been shown to be an independent risk factor for developing chemotherapy resistant AML in our study.

4.12. Survival of patients with AML

The overall survival of patients with AML in this study was dismal, with a median survival time of 4.8 months (95% confidence interval of 0.2-0.7 years). The poor survival is largely a reflection of the significant number of early deaths, within the first two to three months. Delays in referral, with more advanced disease at presentation are a major contributor in this group of patients with AML. This is supported by the improved survival rates when the survival analysis is performed excluding this group of patients with early mortality. There is a two-fold increase in the 1-year, 3-year and 5-year survival, respectively. There was however, no difference in survival rates between the HIV seronegative and HIV seropositive AML patients with a p-value of 0.4 (including early mortalities) and 0.98 (excluding early mortalities). Although there are many factors that may influence the survival of AML patients, it cannot be denied that resource limitations and general access to health care facilities remain ongoing challenges in the treatment of AML in the South African context.

4.13. Limitations of the study

- Due to the retrospective nature of this study, the data collected was limited by missing and incomplete clinical and laboratory patient records.
- Approximately 18% of the patients were lost to follow up.
- The sample size of HIV seropositive patients was small, with only 33 patients being evaluated. This is primarily due to the rarity of AML occurring in HIV seropositive patients.

CHAPTER 5: CONCLUSION

5.1. Conclusion

After the lymphomas and multiple myeloma, Acute Myeloid Leukaemia is the next most common haematological malignancy encountered in adults at Chris Hani Baragwanath Academic Hospital. Although HIV infection is seen in pandemic proportions at CHBAH, the association of AML and HIV infection is a rare occurrence. Nevertheless, the 33 HIV seropositive patients with AML in our study constitute the largest number of patients with this association diagnosed at a single center. However, direct comparisons were only made with regard to 27 HIV seropositive patients compared to HIV seronegative AML patients, during the period 01/01/2005 to 31/12/2014. HIV seropositive patients with AML present at a younger age, with a female predominance. The clinical presentation is similar to that described in the literature with symptoms and signs of bone marrow failure/infiltration.

In general, the HIV seropositive patients with AML had more profound cytopenias and a lower albumin level compared to their HIV seronegative counterparts. AML (M2) and AML (M3) were the most frequently encountered morphological subtypes of AML.

The therapy of HIV seropositive patients with AML was similar to that in the HIV seronegative AML patients, with regard to both supportive as well as specific therapy. The HIV seropositive patients were more likely to receive a shorter course of cytosine arabinoside than their HIV seronegative counterparts. Complete remission was achieved in 60.7% of patients receiving induction chemotherapy. The HIV seropositive and HIV seronegative patients had similar remission rates. Chemotherapy resistant disease was present in 38% of the study population; the most prominent risk factor for refractory disease was secondary AML. HIV infection was not an independent risk factor for chemotherapy resistant disease.

Overall the patient outcomes were as follows: 76% of the patients demised, 18% of patients were lost to follow up, and 6% were alive. The survival of AML patients at 6

months was 47% and 32.5% at 12 months. Patients who demised within the first 3 months and who presented with advanced disease at presentation influenced the survival rates. Patient survival improved to 86.7% at 6 months and 60.1% at 12 months, respectively, when the patients with early mortality were excluded.

The current evidence suggests that acute myeloid leukaemia is a co-incidental malignancy in the setting of HIV infection, unlike high grade, B-cell Non-Hodgkin lymphoma, which is an AIDS defining malignancy. Despite there being no definite causal relationship between HIV and acute myeloid leukaemia, it is important to be aware of this association as there are differences between AML in seronegative and seropositive patients. Although similar chemotherapy is offered to both HIV seronegative and HIV seropositive patients, antiretroviral therapy is an important cornerstone of therapy in HIV seropositive patients with AML.

We recommend future prospective studies with a greater focus on the aetiology, biology, clinical presentation, therapy, molecular and genetic characteristics of AML, in this uncommon population of HIV seropositivity coexisting with AML.

REFERENCES

1. Estey E, Dohner H. Acute myeloid leukaemia. Lancet. 2006;368(9550):1894-907.
2. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-47.
3. Dohner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. New England Journal of Medicine. 2015;373(12):1136-52.
4. Fernandez HF. New trends in the standard of care for initial therapy of acute myeloid leukemia. Education Program of the American Society of Hematology. 2010;2010:56-61.
5. Wang ES. Treating acute myeloid leukemia in older adults. Education Program of the American Society of Hematology. 2014;2014(1):14-20.
6. Marshall RC, Tlagadi A, Bronze M, Kana V, Naidoo S, Wiggill TM, et al. Lower frequency of NPM1 and FLT3-ITD mutations in a South African adult de novo AML cohort. International Journal of Laboratory Hematology. 2014;36(6):656-64.
7. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
9. Stone RM. Prognostic factors in AML in relation to abnormal karyotype. Best Practice & Research in Clinical Haematology. 2009;22(4):523-8.
10. Pfirrmann M, Ehninger G, Thiede C, Bornhauser M, Kramer M, Rollig C, et al. Prediction of post-remission survival in acute myeloid leukaemia: a post-hoc analysis of the AML96 trial. The Lancet Oncology. 2012;13(2):207-14.
11. Kansal R. Acute myeloid leukemia in the era of precision medicine: recent advances in diagnostic classification and risk stratification. Cancer Biology & Medicine. 2016;13(1):41-54.
12. Cannas G, Thomas X. Supportive care in patients with acute leukaemia: historical perspectives. Blood Transfusion. 2015;13(2):205-20.
13. Fernandez HF. What Is the Optimal Induction Therapy for Younger Fit Patients With AML? Current Hematologic Malignancy Reports. 2016;11(5):327-32.
14. Canzoniero JV, Bhatnagar B, Baer MR, Gojo I. Upfront therapy of acute myeloid leukemia. Current Oncology Reports. 2011;13(5):361-70.
15. Fernandez HF, Sun Z, Yao X, Litzow MR, Luger SM, Paietta EM, et al. Anthracycline dose intensification in acute myeloid leukemia. New England Journal of Medicine. 2009;361(13):1249-59.
16. Rees JK, Gray RG, Wheatley K. Dose intensification in acute myeloid leukaemia: greater effectiveness at lower cost. Principal report of the Medical Research Council's AML9 study. MRC Leukaemia in Adults Working Party. British Journal of Haematology. 1996;94(1):89-98.

17. Luskin MR, Lee JW, Fernandez HF, Abdel-Wahab O, Bennett JM, Ketterling RP, et al. Benefit of high-dose daunorubicin in AML induction extends across cytogenetic and molecular groups. Blood. 2016;127(12):1551-8.
18. Reagan JL, Sullivan MR, Winer ES, Lansigan F, Cardin MS, Castillo JJ. Potential for improved survival with intensification of daunorubicin based induction chemotherapy in acute myeloid leukemia patients who do not receive transplant: A multicenter retrospective study. Leukemia Research. 2015;39(8):812-7.
19. Novitzky N, Thomas V, Abrahams L, du Toit C, McDonald A. Increasing dose intensity of anthracycline antibiotics improves outcome in patients with acute myelogenous leukemia. American Journal of Hematology. 2004;76(4):319-29.
20. Weisburg JH. Multidrug resistance in acute myeloid leukemia: potential new therapeutics. Journal of Nuclear Medicine. 2008;49(9):1405-7.
21. Estey E. Acute Myeloid Leukemia - Many Diseases, Many Treatments. New England Journal of Medicine. 2016;375(21):2094-5.
22. Short NJ, Ravandi F. Acute Myeloid Leukemia: Past, Present, and Prospects for the Future. Clinical Lymphoma, Myeloma & Leukemia. 2016;16 Suppl:S25-9.
23. Park H, Youk J, Kim I, Yoon SS, Park S, Lee JO, et al. Comparison of cladribine- and fludarabine-based induction chemotherapy in relapsed or refractory acute myeloid leukaemia. Annals of Hematology. 2016;95(11):1777-86.
24. Bose P, Vachhani P, Cortes JE. Treatment of Relapsed/Refractory Acute Myeloid Leukemia. Current Treatment Options in Oncology. 2017;18(3):17.
25. Novitzky N, Thomas V, du Toit C, McDonald A. Is there a role for autologous stem cell transplantation for patients with acute myelogenous leukemia? A retrospective analysis. Biology of Blood and Marrow Transplantation. 2011;17(6):875-84.
26. McMillan AK, Goldstone AH, Linch DC, Gribben JG, Patterson KG, Richards JD, et al. High-dose chemotherapy and autologous bone marrow transplantation in acute myeloid leukemia. Blood. 1990;76(3):480-8.
27. Pollyea DA, Kohrt HE, Medeiros BC. Acute myeloid leukaemia in the elderly: a review. British Journal of Haematology. 2011;152(5):524-42.
28. Tauro S. The blind men and the AML elephant: can we feel the progress? Blood Cancer Journal. 2016;6:e424.
29. Little RF, Dunleavy K. Update on the treatment of HIV-associated hematologic malignancies. The Education Program of the American Society of Hematology. 2013;2013:382-8.
30. Bernstein WB, Little RF, Wilson WH, Yarchoan R. Acquired immunodeficiency syndrome-related malignancies in the era of highly active antiretroviral therapy. International Journal of Hematology. 2006;84(1):3-11.
31. Drilon AD, Gamboa EO, Koolaee R, Goel A. Acute promyelocytic leukemia in HIV-infected adults: a case report and review of therapeutic considerations. Clinical Lymphoma, Myeloma & Leukemia. 2010;10(5):E47-52.
32. Kudva GC, Maliekel K, Richart JM, Batanian JR, Grosso LE, Sokol-Anderson M, et al. Acute promyelocytic leukemia and HIV-1 infection: case report and review of the literature. American Journal of Hematology. 2004;77(3):287-90.

33. Patel M, Philip V and Fazel F. Human immunodeficiency virus infection and Hodgkin's Lymphoma in South Africa - An emerging problem. Advances in Hematology. 2011,doi:10.1155/2011/578163,2011.
34. Patel M, Philip V, Omar T, Turton D, Candy G, Lakha A, et al. The Impact of Human Immunodeficiency Virus Infection (HIV) on Lymphoma in South Africa. Journal of Cancer Therapy. 2015;6:527-35.
35. Malfitano A, Barbaro G, Perretti A, Barbarini G. Human immunodeficiency virus-associated malignancies: a therapeutic update. Current HIV Research. 2012;10(2):123-32.
36. Aboulafia DM, Meneses M, Ginsberg S, Siegel MS, Howard WW, Dezube BJ. Acute myeloid leukemia in patients infected with HIV-1. AIDS . 2002;16(6):865-76.
37. Bower M, Palmieri C, Dhillon T. AIDS-related malignancies: changing epidemiology and the impact of highly active antiretroviral therapy. Current Opinion in Infectious Diseases. 2006;19(1):14-9.
38. Patel M, Philip V, Fazel F, Lakha A, Vorog A, Ali N, et al. Human immunodeficiency virus infection and chronic myeloid leukemia. Leukemia Research. 2012;36(11):1334-8.
39. Maartens G, Celum C, Lewin SR. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. Lancet. 2014;384(9939):258-71.
40. Sutton L, Guenel P, Tanguy ML, Rio B, Dhedin N, Casassus P, et al. Acute myeloid leukaemia in human immunodeficiency virus-infected adults: epidemiology, treatment feasibility and outcome. British Journal of Haematology. 2001;112(4):900-8.
41. Evans MW, Sung A, Gojo I, Tidwell ML, Greer J, Levis M, et al. Human Immunodeficiency Virus (HIV)-Associated Acute Myeloid Leukemia (AML) in the Era of Highly Active Antiretroviral Therapy (HAART): High Complete Remission Rate, but Poor Tolerance of Consolidation Therapy and Short Disease-Free Survival. Blood. 2009;114:4126.
42. Krishnan A. Stem cell transplantation in HIV-infected patients. Current Opinion in HIV and AIDS. 2009;4(1):11-5.
43. Knops E, Kobbe G, Kaiser R, Lübke N, Dunay G, Fischer J, et al. Treatment of HIV and acute myeloid leukemia by allogeneic CCR5-d32 blood stem cell transplantation,doi:10.1016/j.jcv.2016.08.171
44. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K, et al. Clonal architecture of secondary acute myeloid leukemia. New England Journal of Medicine. 2012;366(12):1090-8.
45. Jacobs P, Wood L. Myelodysplasia and the acute myeloid leukaemias. Hematology. 2002;7(6):325-38.
46. Almond LM, Charalampakis M, Ford SJ, Gourevitch D, Desai A. Myeloid Sarcoma: Presentation, Diagnosis, and Treatment. Clinical Lymphoma, Myeloma & Leukemia. 2017;17(5):263-7.
47. Boulle A, Bock P, Osler M, Cohen K, Channing L, Hilderbrand K, et al. Antiretroviral therapy and early mortality in South Africa. Bulletin of the World Health Organization. 2008;86(9):678-87.
48. Scholl S, Fricke HJ, Sayer HG, Hoffken K. Clinical implications of molecular genetic aberrations in acute myeloid leukemia. Journal of Cancer Research and Clinical Oncology. 2009;135(4):491-505.

Appendix A: Data collection sheet

Patient case number: _____

Date of birth: _____ Age:_____

Gender__M_____F_____ Ethnic group:__B__W__C____I_

Date of disease first documented (blasts on peripheral smear/BM/flow cytometry):_____Y_____N

Clinical presentation (at diagnosis)

	Yes	No	If yes, provide details (onset, duration, severity)
Anaemia			
Symptoms of anaemia			
Bleeding (site)			
Skin: easy bruising			
Epistaxis			
Gum bleeding			
Haemoptysis			
Haematemesis			
Malaena			
Haematuria			
Menorrhagia			
Infection			
Fever			
Source of sepsis			
• Respiratory • Genitourinary • GIT • Skin • Musculoskeletal • CNS			
Other			

Night sweats			
Bone pain			
Previous history of malignancy			
Previous exposure to cytotoxic drugs			
Relevant drug history			
Occupational exposure: e.g. benzene, organic solvents			

HIV sero-positive patients

Date of diagnosis: Day_____ Month _____ Year_____

Baseline CD_4 count:_____

Clinical stage: _____

On ART: Y N

If yes,

Duration on therapy (mths) <6 6-12 12-24 24-36 36-48 48-60 >60

Regimen changed: Y N

If yes: detail_____

Clinical examination (at diagnosis)

General	Yes	No	If yes, provide details (where relevant: duration, site, size, characteristics)
Pallor			
Lymphadenopathy			
Bleeding			
Petechiae			
Purpura			
Ecchymosis			
Haemorrhagic bullae			

Abdomen			
Hepatomegaly			
Splenomegaly			
Abdominal mass			
Extramedullary			
Gum hypertrophy			
Skin • Leukaemia cutis			
Myeloid sarcoma			
Infections			
Site of sepsis • Respiratory • Genitourinary • GIT • Skin • Musculoskeletal • CNS			
Positive blood cultures			
• Gram positive bacteria • Gram negative bacteria • Anaerobic organism • Fungal			
HIV associated opportunistic infections			
Tuberculosis			
Site • Pulmonary TB • TB abdomen • TB Meningitis			

Treatment of AML

Induction	Consolidation							Maintenance	
Cycle 1 Regimen: a. b. c. d.	1	2	3	4	5	6	>6	Y	N
Cycle 2								Y	N
Cycle 3								Y	N
Cycle 4								Y	N
Cycles > 4								Y	N

Outcome

Alive: Y N

Survival (duration from diagnosis-current) _____

Current disease status: remission _Y_____N__ Ongoing disease: Y N

If ongoing disease: duration, remission was sustained_____ , relapse

Death: Y N

Survival (duration from date of diagnosis- date of death):_____

Documented cause/s of death: _____

Lost to follow-up:

Date of last clinic visit:_____

Status of disease at last visit:

- Remission: Relapse:

Appendix B: Results flow chart

Bone Marrow Aspirate &Trephine	At diagnosis	At remission	End of consolidation	Relapse (where relevant)	Maintenance (where relevant)
Morphology					
Cellularity • ♿ • N • △					
Blasts % • <20 • 20-50 • 50-80 • >80 • Specify blast count_____					
Background marrow elements					
Evidence of cellular dysplasia					
FAB subtype • MO • M1 • M2 • M3 • M4 • M5 • M6 • M7					
Flow cytometry • **CD45**					

• **CD 13** • **CD 14** • **CD 15** • **CD19** • **CD 34** • **CD 38** • **HLA DR** • **CD 16** • **CD 66**					
Cytogenetics					
• t (15;17) • t(8;21) • inv(16) • $5q^-$ • 7q- • t(9;22) • complex karyotype • FLT_3 • other					
WCC					
Hb					
Hct					
MCV					
PLT					
Neutro					
Mono					
Lymphs					
Eos					
Baso					
Ferritin					
Vit B12					

Red cell folate					
Na					
K					
Chl					
Urea					
Creatinine					
LDH					
Uric acid					
Ca^{2+}					
Mg					
PO_4					
LFT: Tbili					
Cb					
Tprot					
Albumin					
ALP					
GGT					
AST					
ALT					
HIV • Negative • Positive • unknown					
Other:					
HIV+ Cases					
CD4					
HIVVL (RNA copies/ml)					

Appendix C: Plagiarism 'Turn-it-in' Report

ACUTE MYELOID LEUKAEMIA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

ORIGINALITY REPORT

16%	10%	12%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	wiredspace.wits.ac.za Internet Source	1%
2	www.haematologica.org Internet Source	1%
3	Neoplastic Diseases of the Blood, 2013. Publication	1%
4	Submitted to University of Witwatersrand Student Paper	<1%
5	Charles Feldman, Michael Glatthaar, Rajen Morar, Akhter Goolam Mahomed, Suleiman Kaka, Marlene Cassel, Keith P. Klugman. "Bacteremic Pneumococcal Pneumonia in HIV-Seropositive and HIV-Seronegative Adults", Chest, 1999 Publication	<1%
6	Haematology and Blood Transfusion Hämatologie und Bluttransfusion, 1997. Publication	<1%

Appendix D: Ethics Committee Clearance Certificate

R14/49 Dr Didintle Mokgoko

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141169

NAME: (Principal Investigator)	Dr Didintle Mokgoko
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Acute Myeloid Leukaemia and Human Immunodeficiency Virus Infection at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	28/11/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	29/07/2015

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