AN ANALYSIS OF CYTOGENETIC AND MOLECULAR ABNORMALITIES IN PATIENTS WITH ACUTE MYELOGENOUS LEUKAEMIA AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (2013 – 2016)

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

Johannesburg 2018

DECLARATION

I, Mohith Debising declare that this research report, submitted for the Degree of Master of Medicine in the University of the Witwatersrand, is my own work and has not been previously submitted for any degree or examination at this or any other university.

Candidate Signature: _____

The _____ day of _____, 2019

ABSTRACT

Introduction:

Acute Myeloid Leukaemia (AML) is a heterogenous disease with a relatively low incidence worldwide but a disproportionately high mortality rate. Despite modern advances and improved understanding of the disease the overall survival statistics of those afflicted by AML is still poor. Increasing weight is placed on cytogenetic and molecular analyses of patients' specific malignancies through new research findings, allowing for the improvement in prognostication of these patients. In the past few years, a number of new treatments, targeting these abnormalities, have been approved for use, or are currently in development, in the management of AML. In addition, our improved understanding of the prognosis of this disease based on cytogenetic and molecular mutations now allows us to fast-track patients with a high risk for relapse post induction toward haemopoietic stem cell transplantation.

Aims:

The aim of the study was to review the specific cytogenetic and molecular mutational trends in patients managed at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) as well as in samples submitted to the National Health and Services Laboratory (NHLS) from surrounding hospitals from January 2013 to May 2016. The study also involved a review of management and survival outcomes in AML patients managed at CMJAH.

Methods:

A retrospective review was undertaken of cytogenetic and molecular analyses of bone marrow and peripheral blood specimens in patients diagnosed with AML submitted to the NHLS and a review of patients' files and treatment records of patients managed for AML at CMJAH. The study

analysed two separate data subsets, one cohort (Cohort A) analysing cytogenetic and mutational abnormalities and outcomes in patients treated at CMJAH and the other cohort (Cohort B) analysing only molecular mutational abnormalities for patients from surrounding hospitals.

Results:

Cohort A analysed 65 patients, with a median age at diagnosis of 43 years old (range 16 - 74 years) and found most patients (71%) to have abnormal cytogenetics. The most commonly encountered cytogenetic abnormality was t(8;21) translocation seen in 20% of patients. According to prognostic risk stratification most of these patients fell within the favourable prognostic grouping. For this cohort of 65 patients, only one patient had no records available for review of treatment and outcome. Of the remaining 64 patients, 48 patients (75%) underwent chemotherapy as part of their disease management, with 36 (75%) patients surviving initial treatment. Of those who survived induction therapy, 20 patients (56%) achieved a complete remission (CR). Overall one year survival was found to be 43% (95% confidence interval (CI): 29% - 57%) for the group as a whole with a significant difference (HR=0.34; 95% CI: 0.16 – 0.73; p=0.0058) seen between the survival of those who received chemotherapy and those who were managed conservatively.

Cohort B analysed a total of 188 patients, revealing a median age at diagnosis of 47 years old (range 14 – 85 years). The most commonly encountered mutation was of Nucleophosmin 1 (NPM 1) (37 of 188 patients) followed by (FMS-like tyrosine kinase 3) FLT 3 (34 of 188 patients). Mutations of both NPM 1 and FLT 3 were found to be present in 11 patients (6% of this cohort).

Conclusions:

Our study findings were found to be similar to those seen internationally for upper middle income countries (UMICs) and lower middle income countries (LMICs), yielding similar disease patterns

to those of studies undertaken in Brazil and Malaysia. Moreover, the study confirmed the differences in AML epidemiology seen between high income countries (HICs) and UMICs and LMICs. To illustrate this, the cytogenetic analysis in our study revealed the highest prevalence of patients in the age group 20 – 30 years of age, similar to findings in Brazil and Malaysia. On the other hand, studies in the UK and USA, both HICs, reveal AML as a disease of older people of age 60 years and older. The higher incidence of core binding factor (CBF) AML seen in younger patients was also found in Cohort A (20% of patients positive for t(8;21) cytogenetic abnormality) similar to epidemiology trends in other UMICs and LMICs.

ACKNOWLEDGMENTS

I would like to thank my family for all their support and assistance in this endeavour. I would like to thank my wife, Nirvana Bharuthram and mom, Urvasi Debising for their support. I would also like to thank my father in law, Professor Ramesh Bharuthram for all his assistance in the writing of this dissertation.

I would like to thank Professor Paul Ruff, my supervisor, for all his guidance and assistance with this research project. In addition, I would like to thank him for allowing me access to the records of patients managed by his unit at CMJAH. I would like to thank the NHLS at CMJAH for their support and provision of data. I would like to thank CMJAH and the medical superintendent for their permission to conduct my study at the hospital.

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GLOSSARY OF ABBREVIATIONS AND ACRONYMS

ADP	Adenosine Di-Phosphate
AML	Acute Myeloid/Myelogenous Leukaemia
APL	Acute Promyelocytic Leukaemia
ARF	ADP Ribosylation Factor
ASR	Age Standardized Risk (Incidence per 100,000 population)
ATRA	All-Trans Retinoic Acid
CBF	Core Binding Factor Protein
CEBPA	CCAAT Enhancer Binding Protein Alpha
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
ECOG	Eastern Cooperative Oncology Group (Performance Score)
FAB	French American British AML Classification
FLT 3	FMS-Like Tyrosine Kinase 3
HIC	High Income Country
HiDAC	High Dose Cytarabine
HIV	Human Immunodeficiency Virus
IARC	International Agency for Research on Cancer
LMIC	Low-middle Income Country
NAACCR	North American Association of Central Cancer Registries
NHLS	National Health Laboratory Services
NPM 1	Nucleophosmin 1
SEER	Surveillance, Epidemiology and End Results Program
UMIC	Upper-middle Income Country
WHO	World Health Organization

CHAPTER 1 : INTRODUCTION

1.1 General Introduction

Acute Myeloid Leukaemia (AML) is a heterogeneous disease with a high mortality rate worldwide.[1] Little data exists regarding the epidemiology of this disease in sub Saharan Africa including South Africa, especially pertaining to the specific cytogenetic and molecular mutational abnormality prevalence which forms an important part of the management and prognosis of this disease. The need, therefore, exists to examine the prevalence of these abnormalities in order to better understand the mortality risk in our population and facilitate an improved management plan for this disease which has such a high global mortality rate especially in lower middle income countries (LMICs).

1.2 Aim of the Study

The aim of the study was to examine the prevalence of specific cytogenetic and molecular abnormalities, as well as treatment and outcomes of patients diagnosed with Acute Myeloid Leukaemia at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

1.3 Study Objectives

Primary objective:

> To analyse the trends regarding cytogenetic and molecular abnormalities in samples submitted to the National Health Laboratory Service (NHLS) in patients with AML diagnosed at CMJAH from 2013 – 2016.

Secondary objectives:

a. A review of patients' outcomes post primary induction chemotherapy.

b. A review of molecular mutational abnormalities in samples submitted to the NHLS from hospitals surrounding CMJAH.

1.4 Literature Review

Acute myeloid leukaemia or acute myelogenous leukaemia is an aggressive heterogeneous haematological malignancy with potentially poor outcomes, especially long term.[1,2] The disease involves the clonal expansion of myeloid precursors i.e. myeloblasts within the bone marrow and peripheral blood.[2] Typically its incidence follows a bimodal distribution with peaks during childhood and early adulthood as well as well as late adulthood but may be seen at all ages.[1,2]

In the adult population AML typically presents with a median age at diagnosis of 66 years.[2] In its latest 2018 statistics, GLOBOCAN estimated the worldwide incidence, standardized for age, of leukaemia to be 5.2 per 100,000.[3] Mortality was quoted at an age standardized rate of 3.5 per 100,000.[3] Leukaemia thus represents a small proportion of malignant diseases with a disproportionately large contribution to malignancy related mortality.[1] It is important to note however that GLOBOCAN data which is collated by the International Agency for Research on Cancer (IARC), does not differentiate between the different types of leukaemia.[3] Thus the above statistics are not AML specific.[3]

GLOBOCAN reported a higher incidence of leukaemia (ASR of 6.3) and a higher incidence of leukaemia related mortality (ASR of 3.7) in countries of very high and high developmental indexes compared to those classified as low developmental index with ASRs of 2.9 and 2.7, respectively.[3]

Possible reasons for this trend may be due to a lack of diagnosis and reporting in countries classified as low developmental index countries, longer life expectancy in higher developmental index countries and due to the undifferentiated nature of the leukaemia statistics provided by the IARC by the various Cancer Registries.

In Europe, data collected from the European Cancer Registry showed an annual incidence of AML of 3.7 per 100,000 over the period from 1995 to 2002 throughout 64 European cancer registries.[4] The data found overall survival rates to be 35% and 15% at 1 year and 5 years post diagnosis respectively.[4] Survival rates in specific age groups revealed the lowest 5 year survival rate in those older than 65 at the time of diagnosis. However in the adult population of 25 to 64 years old and 15 to 24 years old the overall survival rates at 5 years were still only 30% and 56% respectively.[4]

Studies in the U.S.A revealed similar survival rates for the period from 1996 to 2002, with an overall 5 year survival rate of 21.7%.[1] Similar to the European study, survival rates were found to be higher in younger population groups. However 5 year survival rate in patients less than 45 years old at diagnosis was still only 48.5%.[1]

More recent data from SEER (Surveillance, Epidemiology and End Results Program) in the U.S.A revealed a 5 year overall survival rate of just 25.9% in those affected by the disease from 2005 to 2011.[5] For the year 2015, the overall data estimated that AML was responsible for only 1.3% of all new cancer cases but caused 1.8% of all cancer related deaths.[5] Data from the NAACCR (North American Association of Central Cancer Registries) from 2011 to 2015 revealed an annual incidence of AML standardized for age of 4.9 per 100,000.[6]

The South African National Cancer Registry (SANCR) revealed an incidence of leukaemia, standardized for age, of 1.8 in males and 1.0 in females in its most recent 2014 statistics.[7] These

incidences were again not specific to AML and referred broadly to all forms of leukaemia, both acute and chronic.[7]

1.5 Background

The clinical presentation of AML classically results from increasing bone marrow infiltration by primitive myeloid cells, due to a failure in maturation to mature cells, with resultant cytopaenias or extramedullary site infiltration with organomegaly and lymphadenopathy.[8] Symptoms represent those caused by anaemia, thrombocytopenia and neutropenia.[8]

Acute promyelocytic leukaemia (APL) typically presents with a consumptive coagulopathy (both bleeding diathesis and thromboembolic phenomenon) and signs and symptoms of disseminated intravascular coagulation (DIC) due to the release of pro-coagulant factors from promyelocyte granules.[8]

Initial investigations required for patients with suspected AML are a peripheral blood sample for haemoglobin (Hb), white blood cell counts (WCC) with differential counts, platelet counts and peripheral blood smear analysis. In addition, bone marrow aspiration and biopsy, flow cytometry analyses, and cytogenetic and molecular analyses are essential.[2]

Standard induction chemotherapy for AML has been cytarabine-based in combination with an anthracycline antibiotic, either daunorubicin or idarubicin, since the late 1970's.[2] Gale et al published a landmark study in 1977 in which patients were treated with a combination therapy of cytarabine, 6-thioguanine and daunorubicin and achieved complete remission in 79% of cases.[9] The trial involved only 28 participants at the time with a further 6 patients added later.[9] The use of 6-thioguanine has since been abandoned in adults due to difficulties associated with the oral route

of administration of the drug in acutely ill patients, although is still used in paediatric patients. Combination therapy with cytarabine and daunorubicin or idarubicin is now the gold standard of treatment and has since been found to induce complete remission in 65% to 75% of young adults (age<60 years) and in 40% to 60% of older adults (age>60 years).[10] Despite positive results in terms of achieving complete remission the overall survival of patients with AML is still poor, as mentioned previously, due to a high incidence of relapse and subsequent treatment failure.

Therapy for patients with APL is currently a combined regimen of cytarabine and daunorubicin or idarubicin hydrochloride in combination with ATRA (All-Trans Retinoic Acid) which enhances myeloid differentiation by overcoming the effects of the abnormal gene product of the t(15;17) translocation of the retinoic acid receptor alpha gene.[2] In addition to the above mentioned therapy, studies have shown efficacy of the compound Arsenic Trioxide in the management of APL. Lo-Coco et al[11] showed the use of combination therapy of ATRA, Arsenic Trioxide, cytarabine and daunorubicin had at the very least non-inferior outcomes, with a trend toward improved patient outcomes. Recently, Burnett et al[12] showed in a phase three trial that the use of Arsenic Trioxide was feasible with good outcomes with regard to induction and maintenance of remission.

Overall prognosis in patients with AML is determined by factors such as age (age>60 associated with poor prognosis), level of white cell count at diagnosis (WCC >100,000 associated with poor prognosis), poor performance status (ECOG PS ≥2), the presence of significant comorbidities and cytogenetic and mutational abnormalities.[2,13] According to Grimwade et al[13] and Grossman et al[14] the most powerful independent prognostic indicators in AML is the patient's specific diagnostic karyotype comprising both cytogenetic and mutational abnormalities.

Two classification systems have been used to separate AML into various subgroups. These systems are the older FAB (French American British) classification system, focusing primarily on

morphology and more recently the WHO (World Health Organization) classification system, which focuses more on chromosomal abnormalities and dysplasia.[2]

The FAB classification divides patients into 8 categories labelled as M0 to M7, with M0 being undifferentiated AML, M1 being AML without maturation, M2 being AML with maturation, M3 being APL, M4 being acute myelomonocytic leukaemia, M5 being acute monocytic leukaemia, M6 being acute erythroblastic leukaemia and M7 pertaining to acute megakaryoblastic leukaemia.[2]

Table 1.1: AML FAB Subtype Classification System

M0	Undifferentiated AML
M1	AML without maturation (poorly differentiated) - >90% blasts
M2	AML with maturation (more differentiated) - <90% blasts
M3	Acute Promyelocytic Leukaemia (APL)
M4	Acute Myelomonocytic Leukaemia
M5a	Acute Monoblastic Leukaemia - >80% blasts
M5b	Acute Monocytic Leukaemia - <80% blasts
M6	Acute Erythroblastic Leukaemia
M7	Acute Megakaryoblastic Leukaemia

Note. Adapted from "Acute Myelogenous Leukaemia and Acute Promyelocytic Leukaemia" by WHO. 2014. Retrieved from http://www.who.int/selection_medicines/committees/expert/20/applications/AML_APL.pdf.

The WHO classification of AML subtypes makes provision for patient history, morphological findings and the presence of specific genetic abnormalities. The genetic abnormalities fall into four primary groups: AML with recurrent cytogenetic abnormalities (AML WRC), AML with myelodysplastic changes (AML MDS), AML therapy related (AML TR) and AML not otherwise specified (AML NOS).[2,15]

Table 1.2: AML WHO Subtype Classification System (2008 Revised Criteria)

AML with recurrent cytogenetic abnormalities (AML WRC)	AML t(8;21); RUNX1-RUNXT1 AML inv(16); CBFB-MYH11 AML t(15;17); PML-RARA AML t(9;11); MLLT3-MLL AML t(6;9); DEK-NUP214 AML inv(3) or t(3;3); RPN1-EVI1 AML t(1;22); RBM15-MKL1 AML with mutated NPM1 AML with mutated CEBPA
AML with myelodysplasia related changes (AML MDS)	Complex karyotypes del(7q), del(5q) del(13q), del(11q), del(9q), del(12p) t(5;12), t(5;17) t(11;16), t(3;21) t(1;3), t(3;5) t(2;11)
Therapy related myeloid neoplasms (AML TR)	
AML not otherwise specified (AML NOS)	AML with minimal differentiation AML without maturation AML with maturation Acute Myelomonocytic Leukaemia Acute Monoblastic Leukaemia Acute Erythroid Leukaemia Acute Megakaryoblastic Leukaemia Acute Basophilic Leukaemia Acute panmyelosis with myelofibrosis
Myeloid Sarcoma	
Myeloid proliferations related to Down Syndrome	
Blastic plasmacytoid dentritic cell neoplasm	

Note. Adapted from "Acute Myelogenous Leukaemia and Acute Promyelocytic Leukaemia" by WHO. 2014. Retrieved from http://www.who.int/selection_medicines/committees/expert/20/applications/AML_APL.pdf.

As mentioned above the presence of cytogenetic abnormalities and molecular mutations have now been identified as the most important markers of prognosis in patients with AML. These cytogenetic and molecular mutational abnormalities are now being used to risk stratify patients with AML into those with favourable, intermediate and poor risk profiles.[13,15–17]

These distinct recurrent cytogenetic abnormalities are found in 50% to 60% of individuals with AML.[18] Patients with a normal genetic karyotype constitute 45% - 55% of the patients with AML.[18] Grimwade et al[13] studied the cytogenetic profile of 5876 patients treated for AML in the UK and found 41% of patients to have a normal karyotype and 46% to have an abnormal karyotype. Of the patients with abnormal karyotypes it was found that 28% had abnormalities classified as recurrent cytogenetic abnormalities and 18% had abnormalities classified as MDS related, in accordance with the WHO classification system.[13] Abnormal karyotypes which have been found to improve prognosis are the following translocational abnormalities: t(8;21) and t(15;17) and inversions in chromosome 16 (inv16) or translocations within chromosome 16.[13,14,19]

The cytogenetic abnormalities t(8;21) and inv(16) result in mutations of the alpha and beta subunits of the core binding factor protein (CBF) and are thus referred to as CBF AML.[20] Within this group of AML, patients may have concurrent mutations in the c-KIT gene which confer a poorer prognosis and higher relapse rate. Up to 50% of CBF AML patients experience a relapse of disease and higher mortality rate.[20]

Abnormal karyotypes resulting in poorer outcomes are deletions in chromosome 5 and 7, inversions and single chromosome translocations involving chromosome 3 and any genetic rearrangement in 11q23 (long arm of chromosome 11) as well as complex karyotypes.[13,14,19] Patients with t(15;17) cytogenetic abnormalities are generally considered as a separate entity as

these patients suffer from APL. Treatment and outcomes are different for this population group as mentioned above and includes the use of ATRA as well as Arsenic Trioxide (As_2O_3).

Within the population of patients with both normal and abnormal karyotypes exist patients with mutational abnormalities which are found to play an important role in risk stratification. Multiple mutational abnormalities have been identified, however the significance of most of these mutations are unclear with regard to prognosis.[15] Mutations found to play an important role in risk stratification are those of NPM1 (Nucleophosmin 1), FLT3 (FMS-like tyrosine kinase 3) and CEBPA (CCAAT enhancer binding protein *a*).[15,16] Of these mutations, Grossman et al[14] and Verhaak[21] et al found mutations in NPM1 to be the most frequent in terms of incidence.[13,21] Mutations in FLT3 were the second most commonly found mutational abnormality with mutations in CEBPA the least frequent in the group.[14,22] Grossman et al[14] further divided CEBPA mutations into single and double mutations, which were found to be in 33% and 67% of the positive cases, respectively. With regard to prognosis, studies have shown an improved prognosis, with increased rates of remission achieved and sustained in patients with NPM1 and bi-allelic CEBPA mutations.[14,21] Conversely, mutations of FLT3 were found to predict lower rates of remission achieved and increased rates of relapse with an overall poorer prognosis.[22]

ADP Ribosylation Factor (ARF) is a tumour suppressor protein found within the nucleolus of all cells capable of inducing cell growth arrest and induction of apoptosis.[23] This protein is found in close proximity to Nucleophosmin within the cell and it is believed that mutations in NPM 1 affect both the location of ARF, moving it from the nucleus to the cytosol, and the breakdown of ARF thus inhibiting its tumour suppressor effect.[24]

FLT 3 is a cell surface receptor which binds to circulating growth factors and plays an important part in early stem cell survival and maturation of cells of myeloid origin.[25] There are two different

forms of FLT 3 abnormalities that have been identified, namely, ITD (internal tandem duplication) and mutations of the TKD (tyrosine kinase domain) subtypes.[25] Abnormalities of FLT 3 result in constitutive activation of the protein resulting in abnormal cell growth and differentiation.[25] To date, only the FLT-ITD subtype have been found to be associated with poorer outcomes.[25]

The CEBPA gene codes for the protein CCAAT enhancer-binding protein alpha, a transcription factor with a tumour suppressor effect.[26] Mutations of this protein impair its ability to bind with DNA and regulate gene expression.[26] As mentioned previously, only bi-allelic mutations of this protein have been associated with improved outcomes in AML.

Table 1.3: AML Cytogenetic Prognostic Factors

Prognostic Risk	Cytogenetic Indicators	Molecular Abnormalities
Favourable Prognosis	Inv(16) or t(16;16) t(8;21) t(15;17)	Isolated NPM1 mutation (absence of FLT3) Bi-allelic CEBPA mutation RAR-alpha translocation
Intermediate Prognosis	Normal cytogenetics Trisomy 8 t(9;11) Others not identified	t(8;21) or Inv(16) with c-KIT mutation
Unfavourable Prognosis	Complex karyotypes Monosomy 5, 5q- Monosomy 7, 7q- 11q23 genetic rearrangements Inv(3) t(6;9) t(9;22)	FLT3-ITD mutation

Note. Adapted from "Molecular Genetic Markers in Acute Myeloid Leukaemia" by S.Yohe. 2015. *J. Clin. Med,* 4(3), 462.

With regard to management, induction therapy gold standard remains cytarabine and anthracycline dual therapy. Bloomfield et al[27] showed in 1998 that the use of high dose cytarabine as consolidation treatment has an independent impact on attaining long term remission. However, these benefits were improved in patients with favourable cytogenetic subtypes.[27] It is important to note that no new treatment modalities were approved primarily for the management of AML from the year 2000 to 2017.[28] Given our new understanding for the molecular basis of the disease and the part these mutations play in leukaemic cell proliferation, there has been significant research since 2000 looking at drugs to assist management of the disease by targeting these specific molecular abnormalities, specifically FLT 3 mutations.[28] Numerous polyspecific tyrosine kinase inhibitors have been used in trials targeting both c-KIT mutations and FLT 3 mutations and the upregulated tyrosine kinases these mutations produce.[29] Several of these tyrosine kinase inhibitors, including the drug Midostaurin were approved for use by the US FDA (Food and Drug Association) in 2017. These first generation tyrosine kinase inhibitors have a low specificity with regard to inhibition of FLT 3, now however a second generation FLT 3 inhibitor, quizartinib is currently undergoing clinical trials and was granted "Breakthrough Designation Status" by FDA in August 2018.[28]

In addition to tyrosine kinase inhibitors, Gemtuzumab ozogomicin, a calicheamicin (enediyne anti-tumour antibiotic derived from a bacterium: Micromonospora echinospora) toxin labelled monoclonal antibody against CD 33 (cluster of differentiation 33), a molecular marker present in certain cases of AML, was previously used in the early 2000's but taken off the market in 2010 following research findings that suggested a low benefit to risk ratio especially in refractory patients.[30] However, as of late 2017 the FDA has again approved Gemtuzumab ozogomicin for the management of AML.[30]

Studies have also shown greater benefit with early allogeneic stem cell transplant over high dose cytarabine (HiDAC) in patients with proven poor genetic risk factors in whom remission is achieved.[10,31] Allogeneic stem cell transplant offers the best means of preventing relapse in AML.[32] This form of consolidation treatment is however associated with significantly higher therapy related morbidity and mortality.[32] Studies have shown no difference between HiDAC and allogeneic stem cell transplant in patients with favourable cytogenetics. However, in patients with intermediate and unfavourable cytogenetics there was a clear benefit demonstrated in patients below the age of 40, thus making the routine testing for both cytogenetic and molecular mutations of paramount importance.[32] Most stem cell transplantation analysis studies utilized sibling donor samples. It has to be noted that it has been shown that outcomes from fully matched unrelated donors are similar to those from matched related donors. Therefore, consolidation with allogeneic stem cell transplant should be considered in all patients under the age of 40 with intermediate or unfavourable risk factors.[32]

It is now well established that AML is a malignancy with a high mortality rate in all age groups. Our understanding of this disease is being continuously updated through the advances in genetic analysis. The associated genetic abnormalities play a clear role in disease response to treatment and recurrence and it is therefore important to study their prevalence in our population group in order to better risk stratify our patients. This study will look at the incidence of these genetic abnormalities in patients with AML diagnosed at NHLS CMJAH and compare the trends to international figures.

CHAPTER 2 : METHODS

2.1 Study Design

The study involved a retrospective review of cytogenetic and molecular abnormalities based on an analysis of peripheral and bone marrow blood samples of patients with AML that were submitted to the National Health Services Laboratory. Additionally, patient hospitalization and management records were reviewed and recorded for analysis using patient file records, both computer and microfiche based. Unfortunately, mutational analysis was requested in only 6 of the 65 patients managed at CMJAH oncology department. For this reason a second cohort was created, reviewing only mutational analysis data, including all samples submitted for analysis from hospitals other than CMJAH. The first set of data, the CMJAH patient cohort (Cohort A), included all patients managed at CMJAH and included all cytogenetic and molecular abnormalities, treatment protocols, remission rates and survival statistics for this group. The second set of data, the non-CMJAH patient cohort (Cohort B) included all specimens submitted to the NHLS for assessment of molecular mutations only. Patients within this data subset were managed at other hospitals with only their molecular testing done at CMJAH NHLS, for this reason there was no access to this groups cytogenetic analysis, treatment protocols remission rates or survival statistics. Samples within this cohort were received from private hospitals in Gauteng and surrounding areas and from other state institutions namely Chris Hani Baragwanath Academic Hospital (CHBAH) and Steve Biko Academic Hospital (SBAH).

2.2 Study Site

The site of study was Charlotte Maxeke Johannesburg Academic Hospital. Data for Cohort A was obtained from samples submitted from patients admitted to and treated by the adult oncology unit at CMJAH. Data for samples submitted for cytogenetic analysis were obtained from the cytogenetic department of the NHLS and patient files were obtained from the records department of CMJAH. Data for Cohort B was obtained from all samples submitted to the NHLS for mutational analysis only including those submitted from hospitals other than CMJAH, therefore no patient records or disease outcome data was available for this cohort.

2.3 Study Population

The study population inclusion criteria included all patients admitted to CMJAH adult oncology unit and NHLS database. Cohort A included all patients who were admitted to and who underwent treatment at CMJAH adult oncology unit (wards 594 and 485) for AML for the period January 2013 to May 2016 with samples submitted for cytogenetic and molecular analysis. A sample size of 65 patients was obtained for cytogenetic and outcome studies for the time period January 2013 to May 2016. Cohort B was obtained from the NHLS and included all samples submitted for assessment of mutational abnormalities from patients over the age of 16 years with a primary diagnosis of AML only and had a sample size of 188 patients for the time period of January 2013 to May 2016.

Inclusion criteria (Cohort A):

1. Patients with AML admitted to and managed at CMJAH oncology unit.
2. Patients over the age of 16 years old .

3. Patients for the time period January 2013 to May 2016.

Inclusion criteria (Cohort B):

1. Patients with samples submitted to the NHLS at CMJAH for molecular analysis from any institute.
2. Patients over the age of 16 years old.
3. Patients for the time period January 2013 to May 2016.

2.4 Data Collection

Data collected was recorded in spreadsheet format using the tables submitted in the study protocol. All patient data was treated confidentially, and the names of the patients were not disclosed in recording the data. Cohort A recorded patient age, gender, HIV status, FAB classification, WHO classification and karyotype and mutational analysis results if available. Chemotherapeutic protocols and outcomes with regard to remission, relapse and survival for this specific cohort of patients were also recorded for analysis. For outcomes with regard to remission, stringent complete remission criteria (sCR) was used in identifying patients who successfully achieved complete remission post chemotherapy.

Cohort B, as mentioned above included all patient samples submitted for mutational analysis from hospitals other than CMJAH. Data was recorded in a separate table and included all eligible samples submitted to the NHLS. Hence, patient records and outcome data were not available for this subset of data and thus did not form part of the data set for analysis. Data recorded included all samples submitted for molecular analysis included NPM, FLT3 and CEBPA mutational abnormalities.

All data used in the study were recorded and documented solely by the principal investigator.

2.5 Data Analysis

Data analysis was carried out using SAS (Statistical Analysis System) using a 5% ($p<0.05$) level of significance. Data analysis was performed by Dr Petra Gaylard of DMSA (Data Management and Statistical Analysis) and the cost of data analysis was covered by the principal investigator. Categorical data were recorded and expressed in the form of bar charts and analysis of the relationships between the data sets was performed using the Chi Square test (X^2) or the Fisher's Exact test in situations where requirements for the X^2 test could not be met. The X^2 test and Fisher's exact test was used to assess the relationship between gender and FAB, WHO classifications, and also between cytogenetics and whether or not remission was achieved. Continuous variables were summarized by the mean, standard deviation, median and interquartile range, and their distribution illustrated by means of histograms. The analysis of the relationships between these data subsets was performed using the Analysis of Variance test and where data did not meet the requirements of this test, a non-parametric test, the Kruskal Wallis test, was used. The relationship between age and FAB, WHO classifications was assessed by one-way Analysis of Variance (ANOVA). Survival curves were derived using Kaplan-Meier estimation. Survival curves were compared using Cox proportional hazards regression.

CHAPTER 3 : RESULTS

3.1 Cohort A Results

3.1.1 Patient Demographics

A total of 65 patients were treated for Acute Myelogenous Leukaemia by the adult oncology unit at CMJAH from the time period for January 2013 to May 2016. All 65 cases were included in the data set used for evaluation of cytogenetic and molecular abnormalities, treatment and outcomes.

The ages of patients included in the study group ranged from 16 years to 74 years. The median age for the data set was 43 years and mean age was 43.5 years. The highest prevalence was recorded for patients within the categorical group of 20 to 30 years old, with the lowest prevalence falling within the group of 70 to 80 years. The distribution of data is as shown:

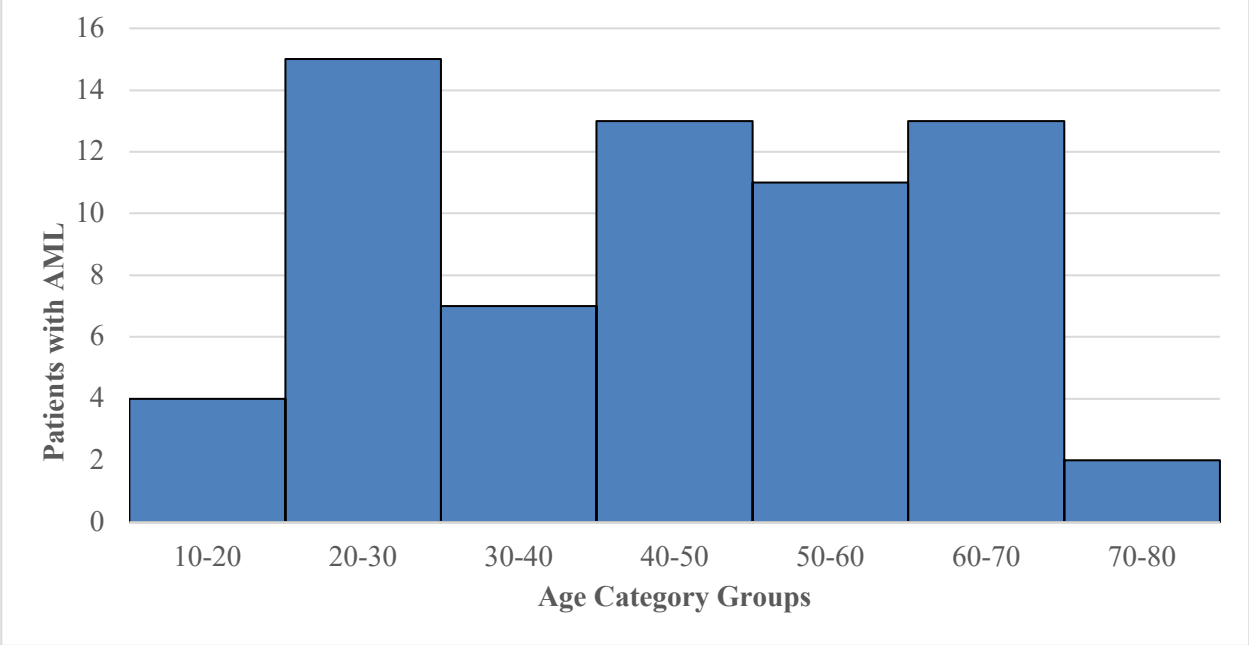

Figure 3.1: Age distribution of Cohort A (n=65)

Analysis of patient gender trends revealed 51% of patients managed at CMJAH for AML during the indicated time period were male and 49% were female.

3.1.2 AML Subtype Classification

All 65 patients managed for AML at CMJAH from January 2013 to May 2016 underwent cytogenetic analysis and subtype classification of their disease according to the FAB classification and WHO classification systems.

The most common subtype using the FAB classification were type M2 and type M4 making up 27.7% and 21.5% of the data set respectively. The least prevalent FAB subtypes were type M5 and type M7, making up 4.6% and 1.5% of the data set respectively. No cases were found to fall under the M6 classification. The graphic below depicts the prevalence of the various FAB subtypes:

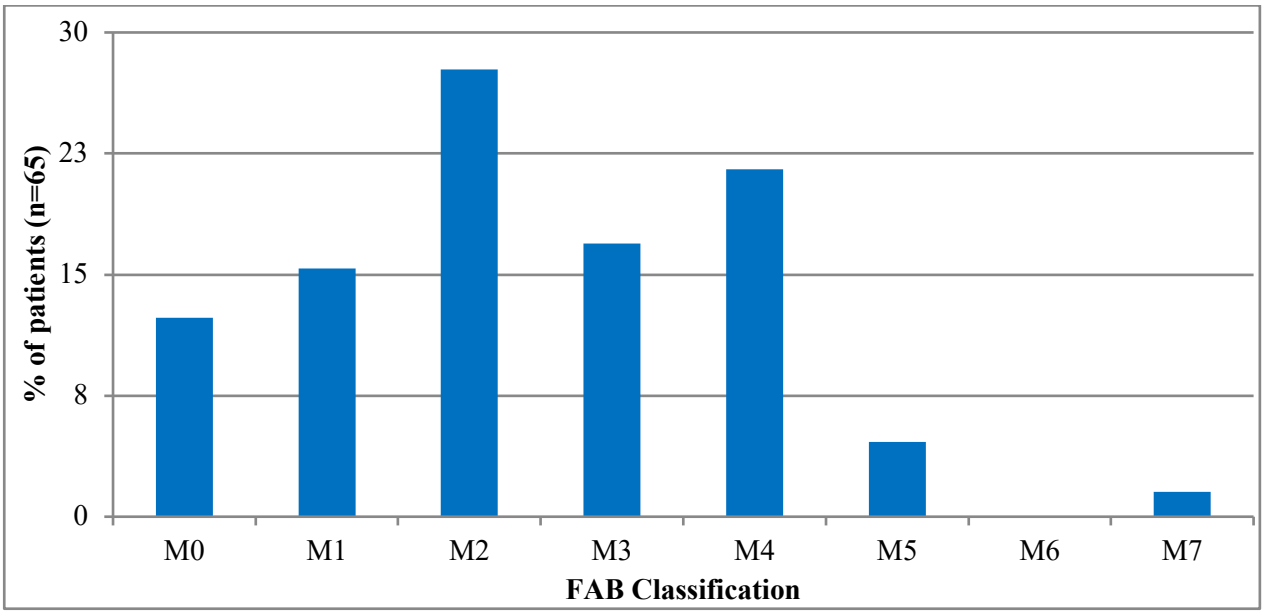

Figure 3.2: FAB Classification based on cytogenetic analysis

Analysis of subtype according to the WHO method revealed a highest prevalence of AML WRC followed by AML NOS with 52.8% and 26.2% of patients falling into these groups respectively. Two patients were classified as AML TR both of whom were previously treated for Acute Lymphoblastic Leukaemia prior to the development of AML. The graphic below depicts the prevalence of the various WHO subtypes:

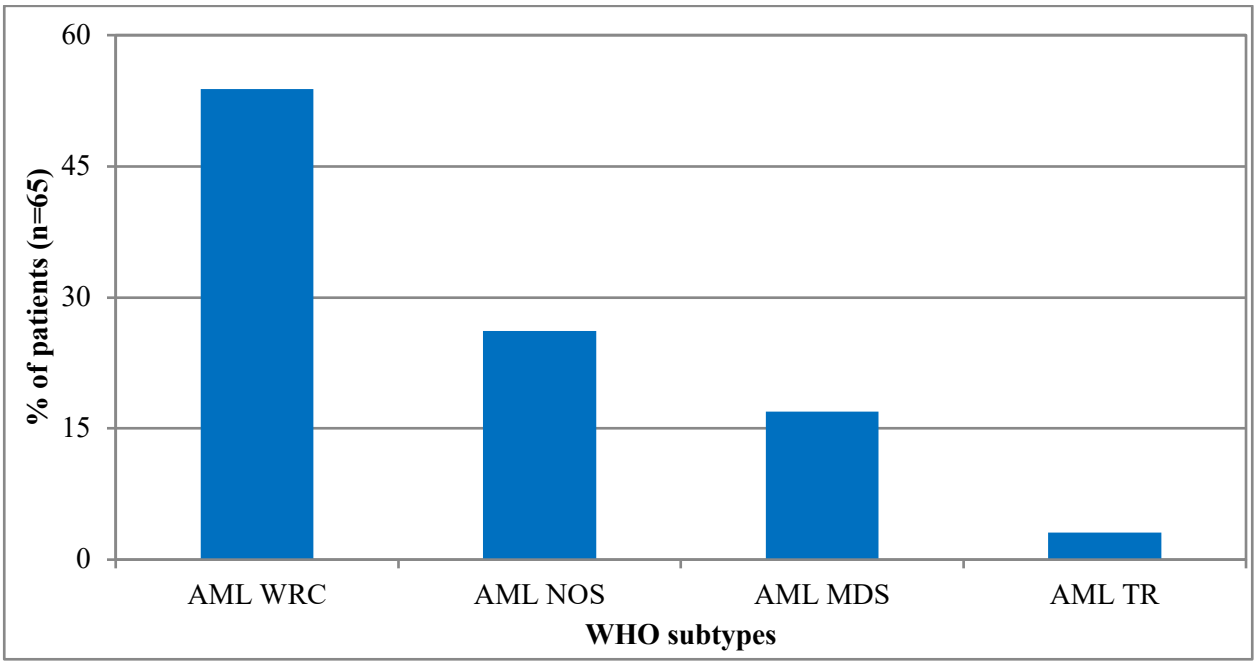

Figure 3.3: WHO Classification based on cytogenetic analysis

3.1.3 Cytogenetic and Molecular Analysis

All molecular mutational testing was performed by the NHLS. The FLT 3, NPM 1 and CEBPA mutations were tested for using a Q-PCR (quantitative PCR) based testing modality using mutational specific probes. Initially testing was only performed for FLT 3 and NPM 1. Testing for mutational abnormalities in CEBPA was instituted in late 2015 (September 2015).

Analysis of samples for mutations and gene re-arrangements was performed for all 65 patients managed for AML and included in the data set. Cytogenetic analysis was performed using both FISH and conventional karyotyping. Of the 65 patients in Cohort A, analysis was performed using both FISH and conventional karyotyping in 55 of the 65 patients. This is important to note as conventional karyotyping analysis allows us to detect abnormalities that may be missed if only FISH with limited probes is utilised. The use of routine karyotyping is especially important in the detection of patients with complex karyotypes.

Of the patients who did not have karyotyping performed on their specimens, 4 of the 10 patients had a sample which was inadequate for assessment and 6 of the 10 patients had no karyotyping requested. Despite the use of routine karyotyping for analysis of most of our specimens, only one patient in Cohort A was found to have had a complex karyotype.

Overall, patients with the presence of single or multiple cytogenetic abnormalities outnumbered patients with normal cytogenetics detected on analysis. Patients with normal cytogenetic analysis made up 29% of the data set and patients with the presence of various cytogenetic abnormalities made up the rest of 71% of cases. The most frequently encountered cytogenetic abnormalities detected were t(8;21) and t(15;17) which were found in 20% and 17% of cases analysed, respectively. Within the subset of patients with cytogenetic abnormalities detected upon analysis of bone marrow samples, it is worth noting that some patents had more than one cytogenetic abnormality present, therefore, the percentages do not sum up to one hundred percent in the graph below depicting the prevalence of the various cytogenetic subtypes detected during bone marrow analysis:

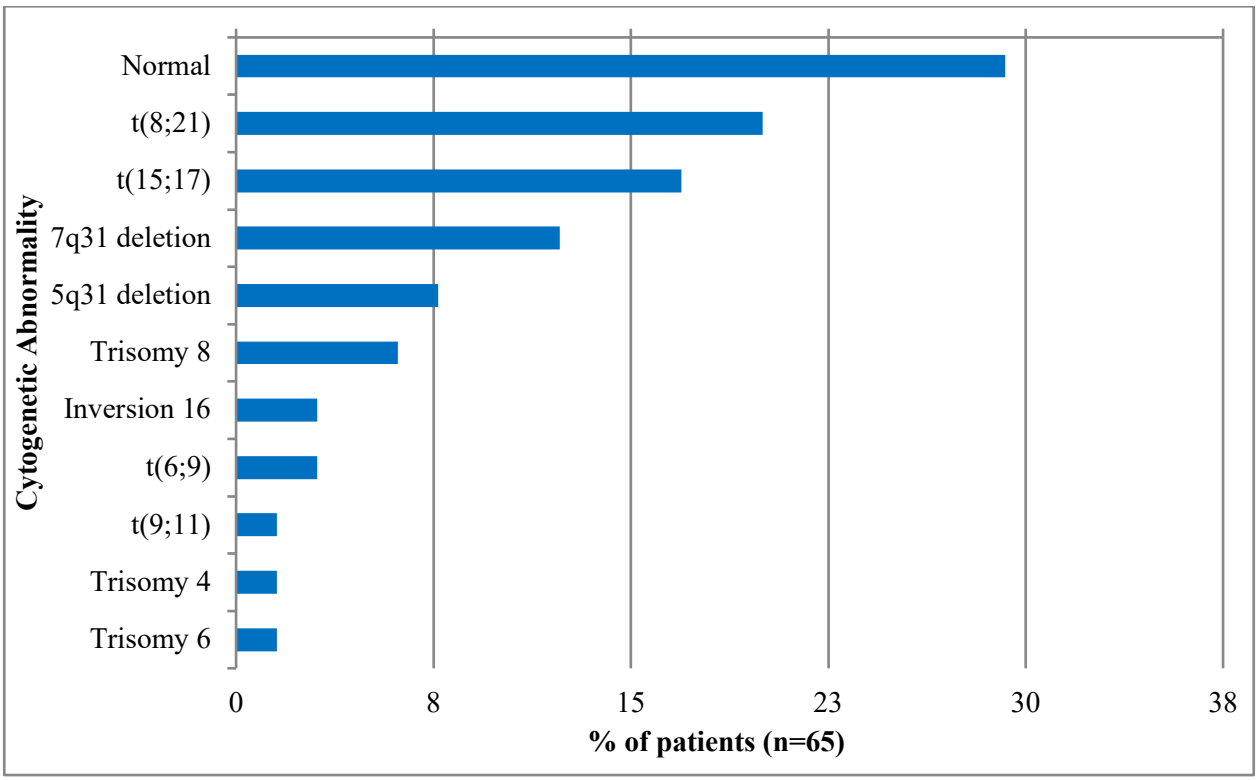

Figure 3.4: Distribution of cytogenetic results obtained

As previously mentioned, molecular mutational analysis was requested in only 6 of the 65 patients managed at CMJAH and included in Cohort A. Of these patients, 4 patients were found to have mutational abnormalities detected with 2 patients testing positive for isolated mutations in FLT 3, 1 patient testing positive for isolated bi-allelic mutations in CEPBA and 1 patient testing positive for mutations in both NPM 1 and FLT3.

3.1.4 Chemotherapeutic Protocols

Of the 65 patients included in the data set, 64 patients had ward records available for review of management plans and outcomes. For one patient, the cytogenetic analysis and diagnostic were available at the NHLS, however no records could be found in the CMJAH records department

regarding the patients' management in the hospital. Of the 64 records reviewed for treatment protocols, 48 patients (75%) underwent chemotherapy after diagnosis. Of these patients 38 (79%) were managed using the combination of cytarabine and daunorubicin or idarubicin hydrochloride, while 9 patients (19%), those with APL, were managed using ATRA and the combination of either daunorubicin or idarubicin hydrochloride and cytarabine. One patient, diagnosed in the department of obstetrics, was initiated on ATRA alone and demised prior to her transfer to the oncology unit. Patients who did not receive chemotherapy either were deemed not fit enough to tolerate treatment, presented with concomitant sepsis precluding chemo and did not survive or refused therapy opting for palliative management. Patient karyotype did not affect patients management in terms of regimen of induction chemotherapy or eligibility for therapy. Patient karyotype was considered post induction therapy in those patients surviving induction chemotherapy. Patients with favourable and normal cytogenetics were potentially eligible for consolidation therapy in the form of cytarabine at a dosage of $2g/m^2$ twice daily on days 1,3 and 5 of therapy. Patients with unfavourable cytogenetics were worked up for allogeneic stem cell transplant but none received this therapeutic modality.

Table 3.1: Chemotherapy

Intervention	Received Chemotherapy (Yes/No)	n	%
Chemotherapy	No	16	25
	Yes	48	75

Induction chemotherapeutic protocols, as mentioned above were the same for all patients deemed medically fit for therapy regardless of their cytogenetic profile. Patients with non-APL type AML received a standardized induction dosing regimen of cytarabine $100mg/m^2$/day on days 1 to 7 of

induction cycle and either idarubicin hydrochloride 12mg/m^2/day on day 5 to 7 or daunorubicin 60mg/m^2/day on day 5 to 70 if the patient's age was <50 years old or >50 years old respectively. Patients found to have a favourable or normal cytogenetic profile who survived induction therapy and achieved remission then went on to receive a consolidation phase of chemotherapy using cytarabine 2g/m^2 every 12 hours on days 1,3 and 5 of consolidation therapy. Patients with APL received 2 cycles of induction therapy with ATRA 45mg/m^2 in 2 divided doses daily on days 1 to 7 and either idarubicin hydrochloride or daunorubicin, depending on patient age, in the dosages mentioned above on days 1 to 4 of both induction cycles. For maintenance therapy, patients with APL received ATRA 45mg/m^2 for 15 days every 3 months and Mercaptopurine 100mg/m^2/day and Methotrexate 10mg/m^2/week. Arsenic trioxide was not utilised in the management of any patient with APL.

3.1.5 Patient Outcomes

Patient outcomes were reviewed by carefully analysing all patient records and files (ward and clinic notes) and dates of death were recorded if the patient demised in hospital. If the patient did not demise in hospital the last date the patient presented to the CMJAH oncology unit, be it the ward or clinic, was recorded. Due to the small sample sizes, the paucity of long-term data available and most importantly the high mortality rate within the first year of presentation, survival curves were estimated at one year post initial presentation. This is based on recommendations for Kaplan Meier survival plots by Pocock et al[33], recommending that plots be extended for a period of follow up until a reasonable proportion of participants (10 – 20%) are still at risk. Due to our sample size the plot was extended only up until 20% were still at risk.

Data for analysis of patient outcomes post primary induction chemotherapy was complete as all treatment is performed on an inpatient basis and patients who received primary induction chemotherapy remained in hospital until assessed for induction outcomes. As previously mentioned a total of 48 patient underwent primary induction chemotherapy for treatment of their disease. Of these, a total of 36 patients (75% of 48 treated) survived induction chemotherapy. Within this subset of patients who survived primary induction chemotherapy, 20 patients (56% of surviving patients) achieved successful complete remission of their disease. Complete remission was defined as per stringent complete remission (sCR) criteria only in accordance with international standards set by the IWG 2003 (International Working Group).[34] Complete remission post induction therapy in this study was thus defined as a presence of less than 5% myeloblasts in the bone marrow in conjunction with an absence of circulating blasts and haematological recovery on peripheral blood.[34] Haematological recovery was defined as peripheral blood absolute neutrophil count > 1000 cells/uL and platelet count >100,000/uL in a patient not requiring further transfusion of packed red cells.[34]

The survival curve below depicts survival estimates for the entire sample of patients who were managed at CMJAH, both those who received chemotherapy and those who did not. The median overall survival for the sample population is 0.4 year (95% confidence interval of 0.3yr to 1.8yr). The survival estimates at 3 months and 1 year were 64% and 43% respectively.

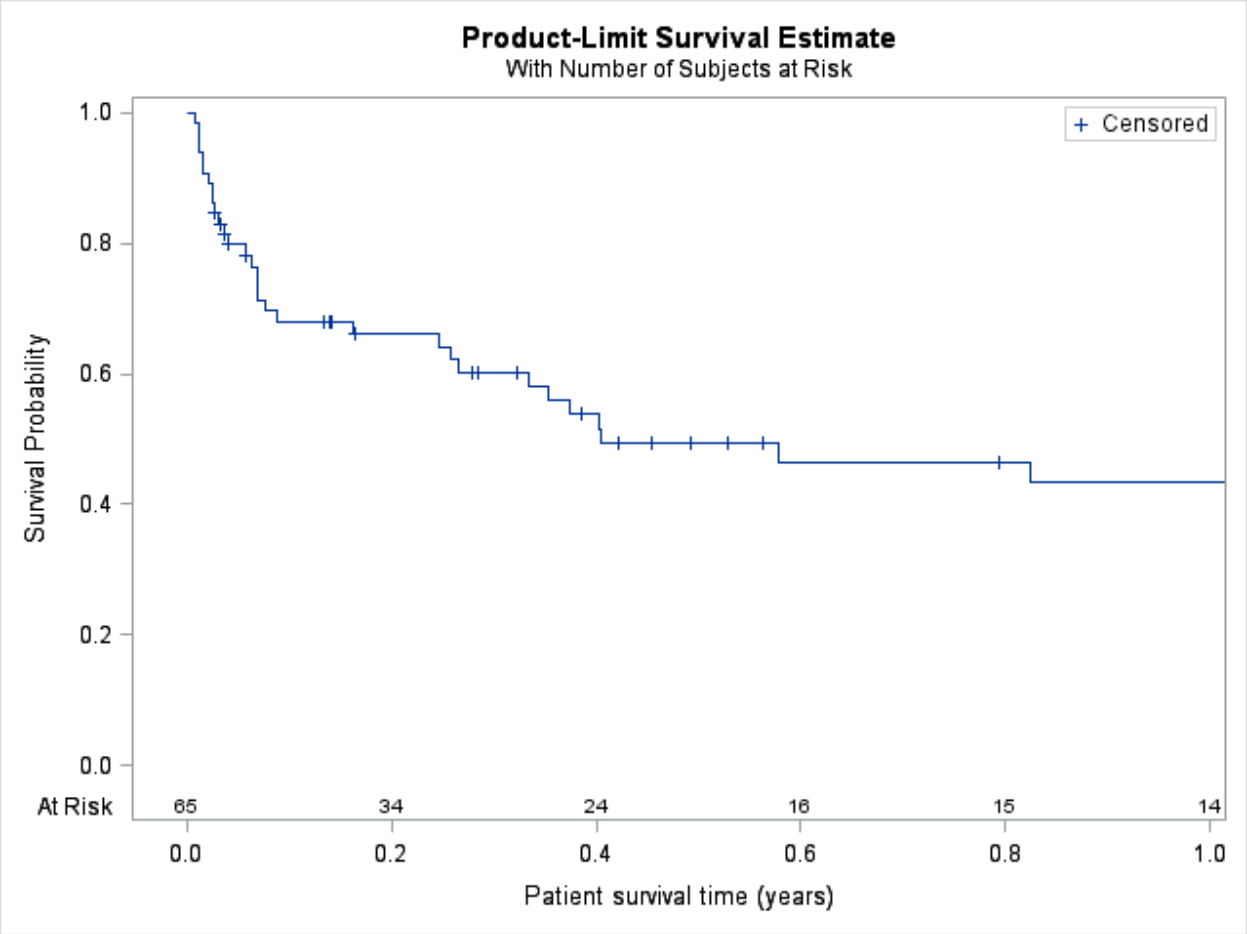

Figure 3.5: Survival estimates for all patients in Cohort A

Table 3.2: Survival estimates for all patients in Cohort A

Time	Survival (%)	95% CI for survival (%)	
3 months	64%	51%	75%
1 year	43%	29%	57%

The survival of patients who underwent chemotherapy compared to those who were conservatively managed was significantly longer (Hazard ratio (HR) 0.34; 95% CI 0.16-0.73; p=0.0058). The survival of patients conservatively managed was 34% at 3 months and 34% at 1 year of follow up. Many of these patients conservatively managed were discharged to undergo palliation at home

thus the data available regarding their mortality is missing, explaining the identical survival statistics at 3 months and 1 year. The one-year survival statistics in the conservatively managed group was most likely falsely elevated due to the missing data regarding mortality in this group. Patients managed with chemotherapy had survival estimates of 72% and 47% at 3 months and 1 year respectively. The survival curve above (Figure 3.5) depicts survival for all patients in Cohort A and the corresponding table (Table 3.2) shows the survival estimates for all patients. The survival curve below (Figure 3.6) depicts survival in patients managed with chemotherapy in Cohort A and includes all patients managed with chemotherapy including those who demised during treatment.

It is important to note that of the original cohort of 65 patients, 23 patients were lost to follow before one year post diagnosis. This represents a significant proportion of patients win whom mortality data is unavailable. This missing data has impacted on our survival curves particularly when comparing patients who had been managed with chemotherapy and those managed conservatively as most patients lost to follow up were those managed palliatively.

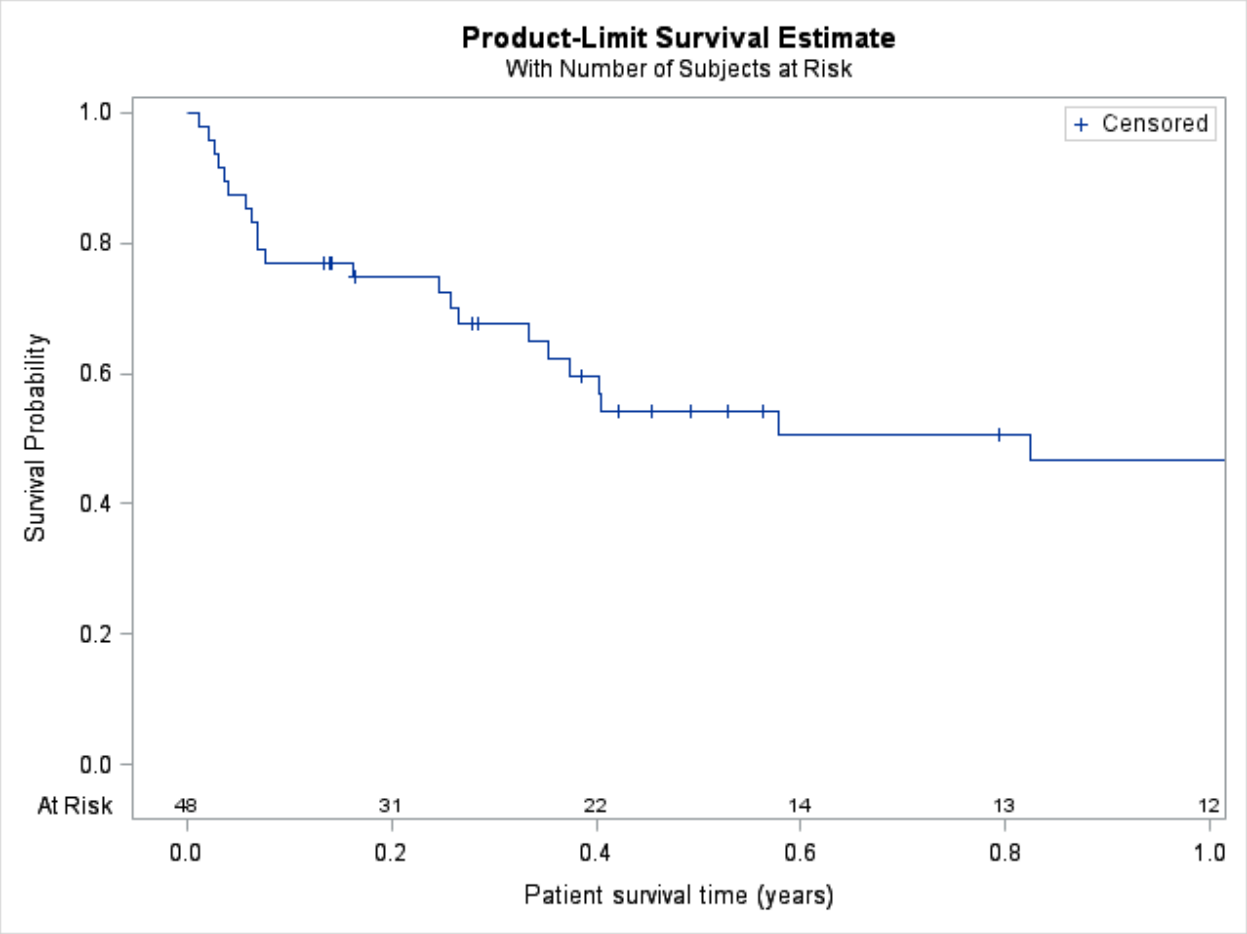

Figure 3.6: Survival estimates of patients managed with chemotherapy in Cohort A

Table 3.3: Survival estimates for patients managed with and without chemotherapy in Cohort A

Time	No chemotherapy			Chemotherapy		
	Survival (%)	95% CI for survival (%)		Survival (%)	95% CI for survival (%)	
3 months	34%	10%	60%	72%	57%	83%
1 year	34%	10%	60%	47%	30%	62%

In terms of remission achieved, there was a significant association between remission achieved and the presence of the t(8;21) cytogenetic abnormality, with remission achieved in all patients

with this abnormality who underwent chemotherapy (p=0.0091). There were no significant associations between any of the other cytogenetic profiles in the data set and remission achieved.

Table 3.4: Cytogenetic and Molecular abnormalities of patients surviving induction chemotherapy including induction outcomes for patients in Cohort A

	Overall	Complete Remission			
		No		Yes	
	n	n	%	n	%
Category	**36**	**16**		**20**	
Normal	8	6	75	2	25
t(8;21)	9	0	0	9	100
t(15;17)	8	1	13	7	88
7q31 deletion	4	3	75	1	25
FLT3	3	3	100	0	0
5q31 deletion	2	2	100	0	0
Trisomy 8	2	2	100	0	0
t(6;9)	2	2	100	0	0
Inversion 16	1	0	0	1	100
t(9;11)	1	1	100	0	0
NPM	1	1	100	0	0

- Note: some patients were diagnosed with more than one abnormality

As depicted in Table 3.4, the number of patients surviving chemotherapy were 36 of the total 48 patients who underwent induction therapy. Of the patients undergoing induction therapy, 12 (25% of total 48 patients) did not survive. The table below (Table 3.5) provides a breakdown of the cytogenetic and mutational abnormalities seen in patients who demised during induction therapy.

Table 3.5: Cytogenetic and molecular abnormalities in patients who demised during induction therapy in Cohort A

Category	Demised during induction therapy	
	Total managed with chemotherapy	Total demised during induction
	n	n
	48	**12**
Normal	11	4
t(8;21)	9	2
t(15;17)	10	2
7q31 deletion	5	3
FLT3	3	0
5q31 deletion	2	1
Trisomy 8	2	0
t(6;9)	2	0
Inversion 16	2	1
t(9;11)	1	0
NPM	1	0

The above table (Table 3.5) depicts an increased survival of induction therapy in patients undergoing chemotherapy having been diagnosed with favourable cytogenetics. Due to the small sample sizes the data presented in Table 3.5 was not of statistical significance. Table 3.6 shows the numbers of patients who successfully achieved remission post induction therapy. As previously mentioned the total number of patients surviving induction therapy was 36 (Total patients treated: 48). Table 3.6 depicts similar data to Table 3.4, however patients are divided in prognostic groups based on their cytogenetic profile. The data clearly shows and increased incidence of remission in patients with favourable cytogenetics. These finding were largely driven by the high incidence of remission achieved in patients with t(8;21) and t(15;17) cytogenetic profiles.

Table 3.6: Prognostic grouping of patients surviving induction chemotherapy including induction outcomes for patients in Cohort A

Prognosis	Induction survivors		Complete remission	
	n	% (of initial 48)	n	%
Favourable prognosis	18	50	17	85
Intermediate prognosis	10	28	2	10
Unfavourable prognosis	8	22	1	5

3.2 Cohort B Results

A total of 188 patients underwent testing for molecular mutational analysis at the NHLS molecular cell genetics department. Of these patients all were tested for NPM 1 and FLT 3 mutations. As discussed previously, testing for molecular mutational abnormalities was performed using a Q-PCR based modality utilising mutational specific probes. CEPBA testing was unfortunately only introduced in late 2015 and very few tests were performed in 2016. Only 13 patients of this second subset of 188 patients were tested for CEBPA, only one of whom tested positive for the bi-allelic mutational abnormality with the rest all testing negative for mutated forms of CEBPA (patients with wild type CEBPA).

For this data set the median patient age was 47 years old and mean age of 47 years old with a range of 14 to 85 years old. A total of 72 patients tested positive for the presence of at least one molecular mutational abnormality. Only one patient tested positive for a bi-allelic mutation of CEBPA, however, only 13 patients were screened for this mutation in the cohort. Of the 188 patients tested, 34 patients were found to be positive for mutations in FLT 3 (18.1% of 188 patients), while 37 patients tested positive for NPM 1 (19.6%% of 188 patients), see Table 3.5 below. Patients found

to be positive for both NPM 1 and FLT 3 made up 5.6% (11 of 188 patients) of the total subgroup and 15.3% of patients positive for any molecular abnormality (11 of 72 patients).

It is important to note that the circumstances surrounding the requests of these tests by the various medical care facilities is unknown. Therefore, we do not know if these tests were requested routinely on all patients or on a select few based on their treatment response and risk factors. Consequently, demographic data in this subset cannot be adequately interpreted.

Table 3.7: Mutational analysis results for patients in Cohort B

Mutation	n (tested)	n (positive)	% (positive)
NPM 1	188	37	19.6
FLT 3	188	34	18.1
CEBPA	13	1	7.7
>1 Abnormality	188	11	5.6

3.3 Summary of the Results

During the period from January 2013 to July 2016 there were a total of 65 patients treated for Acute Myelogenous Leukaemia at the oncology unit at Charlotte Maxeke Johannesburg Academic Hospital, which consisted of 33 males (51%) and 32 females (49%). The median age in the data set was 43 years (range 16-74 years). The highest prevalence of patients fell in the age group 20-30 years old (15 of 65 patients). Of the 65 patients, 58 (89%) of the patients had documented testing for HIV of which 14 patients (24% of 58) were found to be positive. The data derived from these patients was recorded in Cohort A.

For the 65 cases, the most common FAB classification was type M2 and most common WHO classification was AML with recurrent cytogenetic abnormalities. Patients were found to have normal cytogenetics in 29% of cases and some form of cytogenetic abnormality, either single or multiple, in 71% of cases. The most frequently encountered cytogenetic abnormality was the t(8;21) mutation (20% of patients) and t(15;17) mutation (17% of patients), with 29% of patients having normal cytogenetics.

Within Cohort A, 48 patients (75% of patients with available data) received induction chemotherapy with a view to curing the disease. Of these 36 (75% of 48) survived induction chemotherapy and 20 patients (56% of 48) achieved complete remission of their disease.

Survival trends of patients within the cytogenetic analysis subset revealed an overall survival estimate of 43% at 1 year. There was a significant difference in 1 year survival outcomes between patients who underwent chemotherapy and those who did not (47% vs 34%; Hazard ratio 0.34; 95% CI 0.16-0.73; p=0.0058)

Cohort B contained a total of 188 patients, who had molecular mutational analysis performed at the NHLS from hospitals other than CMJAH, both private and state institutions namely CHBAH and SBAH, in the areas surrounding CMJAH. revealing a median and mean age of 47 years old. The mutational abnormalities NPM 1 and FLT 3 occurred virtually equally, being found in 20% 37 of 188 patients) and 18% (34 of 188 patients) of patients respectively. Patients found to be positive for both NPM 1 and FLT 3 made up 5.6% of cases analysed. Unfortunately, testing for CEBPA was only initiated in 2015 and very few requests were made during the period of data collection, thus the data for this abnormality is limited.

Table 3.8: Summary of results (Cohort A)

Variable	Category	n	%
Gender	Female	32	49
	Male	33	51
FAB classification	M0	8	12
	M1	10	15
	M2	18	28
	M3	11	17
	M4	14	22
	M5	3	5
	M6	0	0
	M7	1	2
FAB classification (grouped)	M0-1	18	28
	M2-3	29	45
	M4-7	18	28
WHO classification	AML WRC	35	54
	AML NOS	17	26
	AML MDS	11	17
	AML TR	2	3
Cytogenetic and Mutational analysis	Normal	19	29
	t(8;21)	13	20
	t(15;17)	11	17
	7q31 deletion	8	12
	5q31 deletion	5	8
	Trisomy 8	4	6
	FLT3	3	5
	Inversion 16	2	3
	t(6;9)	2	3
	t(9;11)	1	2
	NPM	1	2
	CEBPA mutation	1	2
	Trisomy 4	1	2
	Trisomy 6	1	2
Chemotherapy (n=64)	No	16	25
	Yes	48	75
Induction survival (n=48)	Demised	12	25
	Survived	36	75
Remission (n=36)	No	16	44
	Yes	20	56

CHAPTER 4 : DISCUSSION

4.1 Discussion of Results

A total of 65 patients were included in the data set for patients managed for Acute Myelogenous Leukaemia at Charlotte Maxeke Johannesburg Academic Hospital (Cohort A). As mentioned previously the distribution of male to female cases was virtually equal with 51% s males and 49% females. This is in keeping with internationally quoted data suggesting a slight, non-statistically significant male predominance in most countries.[1]

Interestingly, the median age in Cohort A was found to be 43 years old, with the majority of patients found to be within the age group of 20 to 30 years old. The median age within Cohort B was found to be 47 years old. Epidemiological data in the United States of America (USA) and United Kingdom (UK) differ greatly from this. Data from studies in both the USA and UK show that AML is primarily a disease of older individuals.[1] For example, in the UK in 2006 the median age at diagnosis was found to be 65 years old with 42.8% of patients diagnosed with this condition older than 65 years of age.[1] In the USA in 2006 the incidence of the disease in people younger than 65 years old was found to be 1.8 per 100000 people while the incidence in people older than 65 years old was found to be 17 per 100000 people.[1]

According to the latest data from the NAACCR, analysing the epidemiological trends in AML in the combined population of North America, shows the highest incidence of new cases of AML in patients aged 80-84 years old (incidence rate of 27.04 per 100,000), with the combined incidence of newly diagnosed cases within the age groups of 20-24 and 25-29 years old only 2.15 cases per 100,000.[6] The Figure 4.1 below, prepared using data from the NAACCR, depicts their trends for AML from 2011-2015.[6]

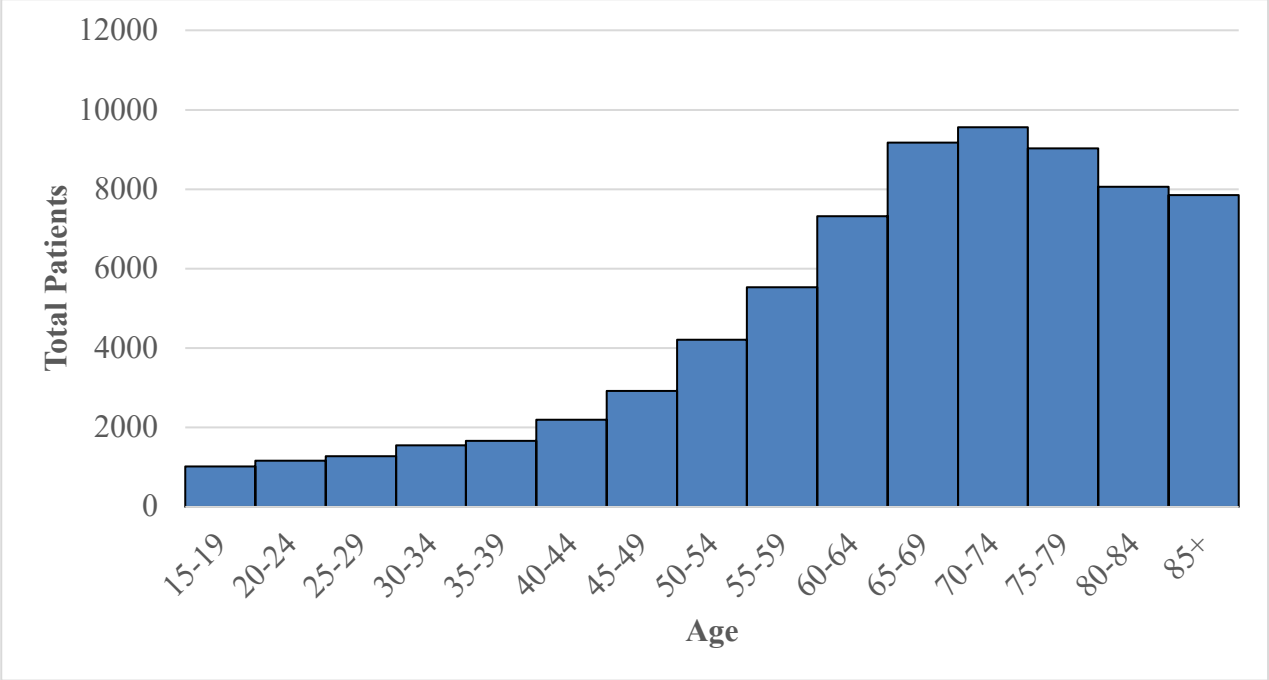

Figure 4.1: Graphical representation of NAACCR epidemiological data

Similar trends are seen in the latest epidemiological data from the UK, based on registries from Cancer Research UK. Their data, obtained from the time period 2013 – 2015 revealed an increasing incidence with age with 41% of new cases diagnosed in patients over the age of 75 years old.[35] This is shown in Figure 4.2.

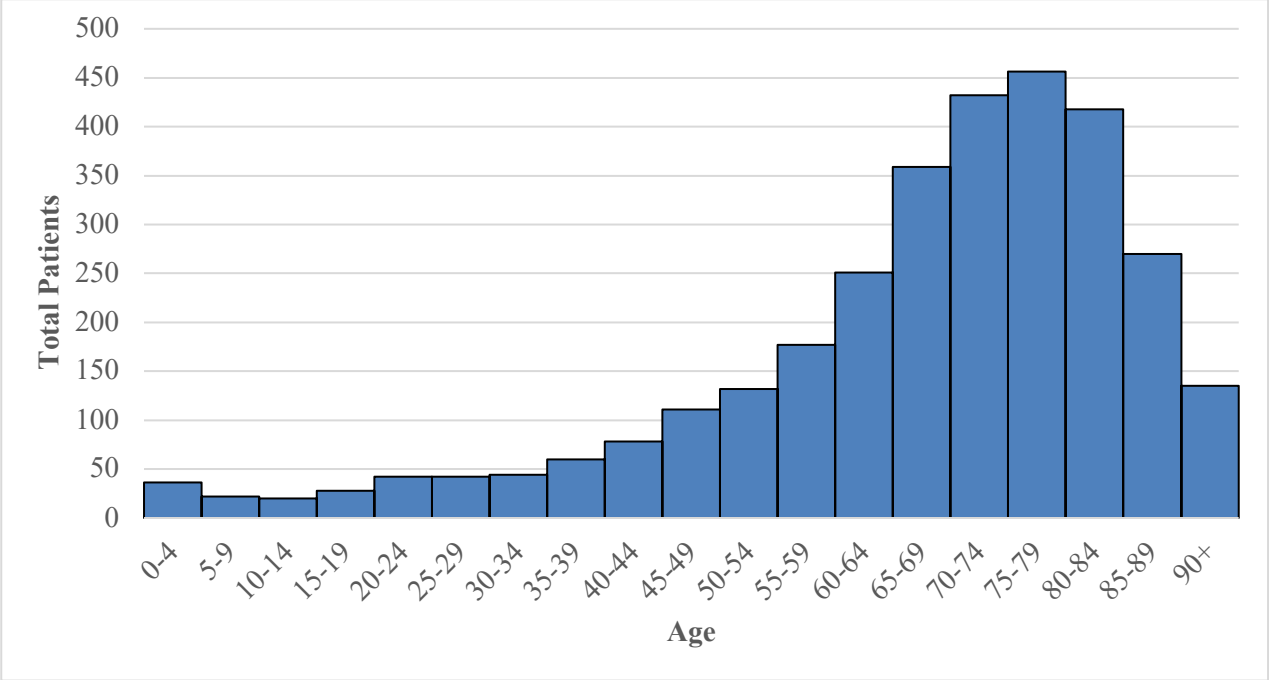

Figure 4.2: Graphical representation of Cancer Research UK registry epidemiological data

Lazarevic et al[19] studied AML cytogenetic trends in Sweden with a cohort of 3251 patients from 1994 – 2006. They found that 58% of their patient cohort had some form of cytogenetic abnormality.[19] Our cytogenetic analysis cohort found cytogenetic abnormalities in 62.8% of patients. In addition, differences emerge in the incidence of the types of cytogenetic abnormalities. In their cohort they found that the most common abnormalities detected were complex karyotypes (24%) and deletions in 7q and 5q (23%).[19] This is in contrast to our data which revealed t(8;21) to be the most commonly encountered cytogenetic abnormality (20% of patients) with the combined deletions in 7q and 5q present in 20% of patients. The most likely reason for the observed differences may be due to the difference in age trends seen between the two sets of data. In the study of Lazarevic et al[19] the data showed the most commonly encountered age group was 70-79 years old, however our data revealed a trend toward a younger population being affected the most

with the commonly seen age group being 20-30 years old. This would explain the number of t(8;21) mutations encountered as this mutation is more typical of younger population groups whereas the AML MDS genetic abnormalities are more common in the elderly thus those mutations were more frequently encountered by Lazarevic et al[19].

As a result of the high incidence of t(8;21) and t(15;17) mutations in Cohort A, most patients were found to have favourable cytogenetics. The table below provides a breakdown of the trends seen in prognostic grouping of patients in our cytogenetic analysis subset.

Table 4.1: Prognostic breakdown of cytogenetic analysis data subset

Prognosis	n	%
Favourable prognosis	26	40
Intermediate prognosis	21	32
Unfavourable prognosis	18	28

The international trends mentioned above, in the UK, USA and Sweden represent trends seen in high income countries (HIC) according to the latest breakdown by the World Bank.[36] South Africa, on the other hand, is currently listed as a upper middle income country.[36] The discussion to follow will analyse trends seen in some other upper middle income countries (UMIC), namely Malaysia and Brazil as well as trends seen in India, a lower middle income country (LMIC).

Meng et al[37] analysed data from 480 patients with AML of all age groups in Malaysia, a similarly classified UMIC, and interestingly found the most frequently affected age group to be 15-30 years old, consistent with our data. They too found the most frequently encountered cytogenetic abnormality to be t(8;21) at 7.5% of total patients, consistent with the younger age of their affected population.[37] The median age of affected individuals in their data was found to be 39 years old, comparable to our median age of 43 years old.[37]

Interestingly, in 2010 Grimwade et al[13] studied a population cohort in the United Kingdom similar in size to that of Lazarevic et al[19] in Sweden, studying cytogenetic trends and outcomes in 5876 patients from May 1988 to January 2009. Their median age at diagnosis was 44 years old with a range of 16 to 59 years old.[13] This younger median age of incidence found again led to the most commonly encountered mutations being t(15;17) and t(8;21), seen in 13% and 7% of patients respectively.[13] This again differs from traditionally quoted epidemiological and demographic data quoted. When compared to the recent Cancer Research UK data, this would seem to suggest a change in the epidemiological trends of AML in the UK over the last 10 years.

With regard to molecular abnormality trend studies, Grossman et al[14] analysed molecular mutation trends in their cohort of 100 patients who were managed for AML with analysis of bone marrow specimens performed at the Munich Leukaemia Laboratory between August 2005 to May 2011. They found NPM 1 mutations to be present in 29.2% of patients, FLT 3 mutations present in 17.9% of patients and CEBPA mutations present in 7.5% of patients (4.4% of total cohort with bi-allelic mutations).[14] Overall survival was also analysed and revealed CEBPA and NPM 1 to be independent positive prognostic factors with regard to patients outcomes.[14]

Falini et al[38] examined 591 specimens in adults diagnosed with AML in Italy and found the presence of the NPM 1 mutation in 208 cases (35% of all cases tested). They also found, in cases

submitted for cytogenetic analysis (493 of 591 cases), most cases with the presence of NPM 1 mutations occurred in the presence of normal cytogenetics (85.% of cases), thus making the presence of the NPM 1 mutation the primary prognostic factor.[38] Verhaak et al[21] submitted 275 cases of AML for cytogenetic analysis and molecular analysis, finding a similar trend with 60% of cases positive for NPM 1 having normal cytogenetics. Interestingly, Verhaak et al[21] also analysed their cohort for relationships between molecular mutations and noted a correlation between the presence of NPM 1 mutations and FLT 3 mutations (60% of FLT 3 positive patients were also positive for NPM 1), noting also a loss of survival benefit in the patient group with both mutations in keeping with the literature available.

For Cohort B, 188 patients who were analysed for molecular mutations, the results revealed NPM 1 mutations in 20% (37 of 188 patients), which is far less frequently observed than that described in the current literature. FLT 3 mutations were present in 18% (34 of 188 patients) and only 32% (11 of 34 patients) testing positive for FLT 3 mutations were found to also have mutations in NPM 1, meaning 68% of FLT 3 mutation positive patients had isolated FLT 3 mutations, conferring a poor prognosis. As previously mentioned, we do not have access to the circumstances surrounding the requests for these tests. Molecular analysis may have been requested routinely or may have been requested only in patients with poor response to induction chemotherapy thus explaining the lower incidence of NPM 1 mutations and higher incidence of FLT 3 single mutations.

Data from RARECARE taking into account 46 regions in Europe shows an overall observed survival at one year of 35% in patients with AML.[4] Survival was significantly higher in patients with APL at 66% at one year.[4] Data from SEER looking at survival in 18 regions within the USA for the period 2008 to 2014 showed a 5 year relative survival for patients with AML to be 28.1%.[39]

Cohort A showed a one year survival estimate of 43% for the group as a whole. There was a significant difference between the outcomes of patients who underwent chemotherapy (one year survival of 47%) and those who did not (one year survival of 34%). As previously mentioned, much of the mortality data for patients who were managed conservatively was missing thus the actual one year survival is likely much lower in this group. In our data 23 of 65 patients were lost to follow up before the one year time period post diagnosis, this would have impacted significantly on our survival statistics.

Looking at epidemiology and outcomes from India, a LMIC, Philip et al[40] found in their cohort of 380 patients, a median age of newly diagnosed patients of 40 years old. They found that 24.7% of patients who underwent induction chemotherapy demised during treatment.[40] This is similar to the mortality statistics in our group with 25% of patients demising during treatment. The cohort of Philip et al[40] did however exhibit superior outcomes with a one ear survival of 55.6% in patients between the ages of 15 to 60 years old and 42.4% in patients older than 60 years old. The patient age statistics were similar to those seen in our cohort with the median age at diagnosis 40 years old.[40] Prognostic groups in their cohort differed from ours with 11.8%, 70% and 18.2% of patients having a favourable, intermediate and unfavourable prognosis respectively.[40] This in contrast to our cohort where most patients had a favourable prognosis.

Interestingly, as previously mentioned, the median age for Cohort A and B was very different to those seen in HICs. Studies from Malaysia, a UMIC and India, a LMIC had median ages similar to our data in Cohort A and B. Lazarevic et al[19] showed in their data that the most frequently encountered cytogenetic abnormalities in their cohort of patients were complex karyotypes and deletions in chromosome 5 and 7. Data from Meng et al[37] and Philip et al[40] revealed cytogenetic trends similar to those seen in Cohort A with the most frequent abnormality seen being t(8;21),

seen in 24% of patients in the cohort of Philip et al and 7.5% of the cohort of Meng et al cohort. This is in keeping with the general consensus that the t(8;21) abnormality leading to CBF AML is more frequently seen in younger population groups. In addition, it is interesting to note the change in age trends seen in the UK by Grimwade et al[13], documenting a median age similar to our own data of 44 years old from the period of 1988 to 2009, in stark contrast to current age trends in the UK. Grimwade et al[13] also found a high incidence of t(8;21) abnormalities in their younger population group (7.5% of patients).

Lima et al[36] studied patients managed for AML in Brazil, another UMIC, examining the records of 241 patients who underwent treatment for AML.[36] Their epidemiology was similar to our own with a median age of 47 years old (median age of our cytogenetic analysis cohort was 43 years old).[36] Their prognostic grouping breakdown was similar to that seen by Philip et al[40], with the majority of patients found to have cytogenetics indicating an intermediate prognosis (64% of patients) and the least common grouping being those with a favourable prognosis (16% of patients).[36,40] Of the patients who underwent standard induction chemotherapy, 57% of patients achieved complete remission.[36] It is noted that this is almost the same as found in Cohort A, of the patients who underwent and survived standard induction chemotherapy, with 56% of patients achieving complete remission. Overall survival statistics were separated into prognostic groups and found to be a median survival time of 88, 224 and 241 days in the unfavourable, intermediate and favourable prognostic groups respectively.[36] Overall one year survival for the cohort as a whole was estimated at 42%.[36] This is very similar to the overall survival findings in our cohort (43% one year survival).

4.2 Study Limitations

There were limitations regarding data collection and analysis pertaining specifically to the small sample size available for analysis in Cohort A, the lack of data regarding the mortality of conservatively managed patients affecting survival analysis and the lack of state funded molecular analysis samples necessitating the use of two discreet cohorts for analysis. As is common in retrospective studies, missing data, especially pertaining to causes of mortality and loss to follow up impacted on the results obtained, specifically with regard to survival estimates. For those attempting to formulate a similar study, a prospective trial with good follow up and pre-set parameters regarding patients management would be important. In addition any future study must ensure that all significant tests are performed for all patients to avoid, as in this study, the use of separate cohorts with one cohort without access to patient outcome data.

CHAPTER 5 : CONCLUSIONS

AML, as noted previously is a heterogenous disease with a relatively low incidence and high mortality rate. This study revealed some interesting trends regarding the epidemiology and outcomes of patients with AML managed at CMJAH especially the high incidence of younger patients with CBF AML.

Interestingly, the demographic data of both the study's cohorts revealed a median age consistent with data seen in other UMICs and LMICs which is in stark contrast to the trends regarding the age groups affected in HICs. The study also revealed differences with regard to the prognostic profile seen in patients managed at CMJAH with a high percentage of patients in the cytogenetic analysis classified as having a favourable prognosis, this was largely due to the high incidence of t(8;21), inv(16) and t(15;17) mutations seen in our population group.

Outcome data of patients in Cohort A revealed trends similar to those seen in internationally quoted studies in other countries. This was in spite of the higher incidence of favourable prognostic groups seen in our data set. The incidence of remission achieved post induction chemotherapy was similar to other studies analysed and quoted. Overall one year survival estimated in the study was similar to data quoted by studies carried out in other middle income countries.

Analysis of data in the molecular analysis cohort of patients revealed a lower incidence of NPM 1 mutations in our population compared to internationally quoted incidences. The incidence of mutations in FLT 3 was found to be similar to those seen in other studies. Unfortunately, as mentioned previously, the circumstances regarding the request of molecular analysis in patients in our cohort was not known. Without knowing if these tests were requested routinely or requested in cases where treatment failure had occurred, it is not possible to draw any formal conclusions using this data.

Overall our study revealed data similar to those seen internationally for UMICs and LMICs. As discussed above our findings are similar to those of studies in Brazil and Malaysia. Interestingly our study highlighted the differences in epidemiology seen between HICs and both UMICs and LMICs. To illustrate, the cytogenetic analysis in our study revealed the highest prevalence of patients in the age group 20 – 30 years of age, similar to findings in Brazil and Malaysia. On the other hand, studies in the UK and USA, both HICs, reveal AML as a disease of older people of age 60 years and more. I was also found that the incidence of CBF AML, a subtype with a more favourable prognosis, was found more frequently in UMICs and LMICs correlating with the younger population group afflicted with AML in these countries. As with internationally quoted data the prognosis of patients seen in our study was poor.

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APPENDIX 1: Ethics Approval Certificate

R14/49 Dr Mohith Debising

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160850

NAME: (Principal Investigator)	Dr Mohith Debising
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	An Analysis of Cytogenetic and Molecular Abnormalities in Patients with Acute Myeloid Leukemia in CMJAH
DATE CONSIDERED:	26/08/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Paul Ruff
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	31/07/2017

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 Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised 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 to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2: Data collection table 1

	Patient Demographics			AML Subtyping				Treatment			Outcome
Number	Age	Gender	HIV status	Cytogenetic Analysis	FAB Classification	WHO Classification	Chemotherapy	Regimen	Remission	
001										
002										
003										
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008										
009										
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040										

APPENDIX 3: Data collection table 2

	Patient Demographics		Molecular Analysis		
Number	Age	Gender	FLT 3	NPM 1	CEBPA
001					
002					
003					
004					
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APPENDIX 4: Turn It In Report

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