INVESTIGATING THE GENETIC AND EPIGENETIC DETERMINANTS OF ACUTE MYELOID LEUKEMIA (AML) PATHOGENESIS

ABSTRACT

Despite the fact that acute myeloid leukemia is a rare illness, it is linked with a high mortality and morbidity. Microscopic and molecular sequencing approaches may be used to identify cytogenetic and molecular abnormalities that are specific to this organism. In high-income nations, these genetic testing are essential for diagnosis and prognosis. As a result, treatment may be tailored to the specific needs of each patient. Many people in the UK do not have access to these tests, hence no data has been collected on this group of individuals. All patients diagnosed with acute myeloid leukemia in the UK's adult hemato-oncology units have to be screened for chromosomal abnormalities and molecular alterations. The research used a cross-sectional descriptive study with sequential sampling. A total of 10 study participants were selected from among those who satisfied the eligibility requirements and gave their approval or agreed to participate. Metaphase G-banding and next-generation sequencing were performed on peripheral blood samples. The clinical and laboratory data, as well as the patient's social and demographic characteristics, were entered into a research proforma. Due to a tiny sample size, no descriptive analysis was performed. Tables were used to display descriptive statistics. The Ampliseq for Illumina myeloid panel was used to conduct cytogenetic analysis and next generation sequencing on ten individuals. The findings of cytogenetic investigations were not conclusive. Mutations were found in 29 of the most frequently mutated genes. A mutation has been found. There were 3 patients with TP53 mutations that fit the criteria for the ELN group. AML patients at certain UK hospitals were found to contain harmful mutations that provide each patient a unique genetic spectrum and contribute to the variety of illness outcomes across patients, according to the findings of this research. It is impossible to determine the prognostic value of these mutations without cytogenetic investigation and the following outcomes.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Acute myeloid leukemia (AML) is a kind of leukemia that originates from stem and progenitor cells in the bone marrow (Li et al., 2016). These cells undergo continual genetic and epigenetic evolution, as well as clonal diversity, as part of their malignant transformation. As a result, AMLs include a wide variety of malignant cell types. Because of the large number of cytogenetic and somatic mutations seen in leukemia subclone cells, the genomes of these cancers are very difficult to unravel (Puumala et al., 2019).

It has been more than a decade since Ley et al. (2008) revealed the first complete genome sequencing of acute myeloid leukemia (AML). Molecular profiling and risk stratification of patients using next-generation sequencing technologies based on the presence of somatic mutations seen in leukemia have become standard and fundamental parts of AML diagnosis since then (Cai and Levine, 2019). Newly diagnosed or relapsed AML patients may benefit from this mutational data, especially because new targeted medicines for FLT3 and IDH1/2 have been authorized for patients carrying these mutations (Krishnan et al., 2022). Sequencing in the AML sector has yielded a wealth of information regarding the disease's mutational and clonal complexity, as well as the pre-leukaemic phases currently known as clonal hematopoiesis (Krishnan et al., 2022). As these studies have shown, epigenetic aberrations have also been shown to be quite common in AML.

Genes that control the normal hematopoietic process are altered in acute myeloid leukemia cells in a way that is specific to this disease (Krishnan et al., 2022). This explains the disease's wide range of phenotypes (Sadikovic et al., 2008). As a result of understanding the underlying biology of AML, new treatment techniques with individualization and identification of post-

treatment minimum residual illness have been developed. Fifty percent of people under the age of 60 and 20 percent of people over the age of 60 have a five-year survival rate of 50 percent and 20 percent for people who have cancer (Carr and Patnaik, 2020). A groundbreaking new anti-leukemic treatment should be able to destroy the malignant founder clone and its sub-clones, eliminating a possible niche for recurrence. Clinical characteristics, cytochemical tests, bone marrow specimen morphological evaluation, cytogenetic testing, immunophenotyping, and molecular testing are used to identify separate biological subgroups with clinical significance in the WHO classification of AML (Thomas et al., 2016). Sub-groups of AML include Undifferentiated acute myeloblastic leukemia, Acute myeloblastic leukemia with minimal maturation, Acute myeloblastic leukemia with maturation, Acute promyelocytic leukemia (APL), Acute myelomonocytic leukemia, Acute myelomonocytic leukemia with eosinophilia, Acute monocytic leukemia, Acute erythroid leukemia, and Acute megakaryoblastic leukemia. Myelodysplastic-related changes, myeloid neoplasms linked to treatment, AML that is not already described, myeloid sarcoma, and myeloid proliferations linked to down syndrome are all in the 2016 WHO classification of AML.

In the past, the morphological description of acute leukemias was used to classify them, based on the predominant cell type in the bone marrow population and its relationship to the cell's normal equivalent. Only a few cytochemical methods such as enzyme localization, micro-spectrophotometry, micro-incineration, radioautography, and cryo-electron microscopy were used to enhance the light microscopic examination of regularly stained blood and marrow smears in this approach. The etiology, morphology, immunophenotyping, and cytogenetics committees of the World Health Organization developed a categorization system in 2001 that differentiates between AML and other myeloproliferative illnesses (Bakhshi and Georgel, 2020). When 20% or more of the nucleated marrow cells are blast cells, a diagnosis of AML is made. Distinct morphological subtypes and clinical profiles are linked to specific

abnormalities. Translocations of chromosomal DNA usually cause these cytogenetic anomalies, which result in novel (abnormal) protein products from the fusion genes that arise. The cellular dysregulation that results in cancer is thought to be caused by the protein products of these fusion genes (Lauschke et al., 2019). Chromosome abnormalities such as AML is vital in selecting treatment plans and have generated significant independent. There are a number of genes, including FLT3, CEBPA, KIT, ERG, MLL, BAALC, and NPM1, that have been associated with a more favorable prognosis in AML patients (Crowther et al., 2008).

Through the activation of signaling pathways, these alterations promote haematopoietic progenitor proliferation and survival. RUNX1, CEBPA, and RARA are examples of transcription factors that are affected by the second class of mutations (Milosevic and Kralovics, 2013). The accumulation of immature progenitors is hampered by mutations or translocations that impact these processes. The frequent detection of mutations in each of these two groups in AML led Gilliland and Griffin to propose a "double-hit" model of leukemogenesis (Milosevic and Kralovics, 2013). They argue that hematopoietic stem cells may be malignantly transformed by either kind of mutation. If both a class I and class II mutation were present, leukemia would arise. It is true that not all AMLs include mutations that exactly match these two kinds of alterations; hence, the double-hit model has certain limitations. According to recent epigenomic research, the epigenetic and transcriptional programming effects generated by combining class I and II mutations were shown to be unique from those caused by each mutation alone (Baylin and Jones, 2016).

Studies have shown that chromosomal abnormalities in AML have a consistent prognostic value, and cytogenetic analysis should be conducted on all newly diagnosed patients (Baylin and Jones, 2016; Puumala et al., 2019; Bakhshi and Georgel, 2020; and Krishnan et al., 2022). As a result, in many facilities, post-remission treatment strategies are strongly dependent on

the results of cytogenetic analysis. Cytogenetic data has been utilized for molecular breakpoint mapping, enabling the use of more sensitive methods such as fluorescence in situ hybridization and primers for reverse transcriptase polymerase chain reaction to be employed. It is important to note that both of these procedures are not employed for general screening or detailed assessment from the outset (Crowther et al., 2008).

From the earliest stages of life to old age, acute myeloid leukemia (AML) affects people of all ages. However, the number of cases is gradually increasing (Sugimura and Ushijima, 2000). AML is a rare kind of cancer, accounting for about 1% of all cancer cases. About 20,050 persons of all ages have been diagnosed with AML in the United States in 2021, 11,140 men and boys, and 8,910 women and girls (Cancer.net editorial Board, 2022). Among adults and children, AML is the second most often diagnosed form of leukemia. The focus of this thesis is to develop a better understanding of how genetic and epigenetic heterogeneity occur in acute myeloid leukemia (AML), if and how they are connected, and what they contribute to the illness from a clinical perspective.

1.2 Statement of the problem

Despite the fact that treatment outcomes have improved over time, acute myeloid leukemia (AML) still ranks as one of the main causes of mortality (Izzo and Landau, 2016). Despite advancements in treatment-associated mortality, chemo-resistance and post-transplant disease recurrence continue to be among the most challenging aspects of AML management. This heterogeneous clonal illness is the result of successive genetic and epigenetic changes in a malignant myeloid stem cell (Izzo and Landau, 2016). The genetic modifications that these cells have undergone interrupt the normal hematological process, causing the formation of aberrant, poorly differentiated neoplastic cells in the blood and bone marrow (Li et al., 2018).

The World Health Organization recommends genetic testing for all individuals with acute myeloid leukemia. Since it is an expensive and crucial examination, many individuals in the UK cannot afford it. This is a group of young, healthy individuals who may benefit from treatment that is individualized for their particular risk.

As a starting point, this research encourages the development of risk-based therapy for a wider sample of patients, which might lead to better outcomes.

1.3 Rationale

Myeloid leukemia is a fatal condition that is spreading across the United Kingdom (Shah and Rawal, 2019). Numerous studies have shown a connection between various anomalies and acute myeloid leukemia (Zhou et al., 2013; Sun et al., 2018; and Shah and Rawal, 2019). Patients who get chemotherapy and have inherited genes have a poor prognosis because of increased cell proliferation brought on by unchecked activation of tyrosine kinase receptors (KIT D816). In spite of the fact that there have been published studies pertaining to this association, our study will be carried out in the UK to find the KIT D816 polymorphism and its relationship with acute myeloid leukemia patients' prognosis.

This research concentrated on the points where genetic and epigenetic variability meet in AML. When it comes to epigenetic modifier genes, we are going to focus on the effect of mutations that arise early in illness and may drastically alter the cell's epigenome and epigenetic landscape, thereby generating a functional connection between genetic and epigenetic diversity.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Acute Myeloid leukemia

Cancer of the blood and bone marrow is acute myeloid leukemia (AML). In adults, acute leukemia is the most frequent form of the disease. If left untreated, this kind of cancer often progresses rapidly (Bravo et al., 2014). Acute myelogenous leukemia (AML) and acute nonlymphocytic leukemia (ANL) are both forms of AML. Proliferation of blasts in the bone marrow, blood or other tissue is the hallmark of acute myeloid leukemia (AML). There is a tendency for the blasts to be myeloid or monocytic in nature (Bakhshi and Georgel, 2020). As many as 80% of individuals with acute myeloid leukemia (AML) will have chromosomal abnormalities, most of which are caused by translocations, which is the transfer of one part of the chromosome to another (Bakhshi and Georgel, 2020). Cell proliferation is uncontrolled as a consequence of the aberrant oncogene or tumor suppression gene expression caused by the translocation. In certain cases, genetic abnormalities and toxic exposure play a role in the etiology (Ramos et al., 2018). Acute myelogenous leukemias have similar clinical symptoms, however they differ in terms of morphology, immunophenotyping, and cytogenetics (Puumala et al., 2013). Cytochemical staining and morphology are employed to determine the blast population's genetic lineage. Subclassification of leukemias may also be accomplished by the use of electron microscopy (Ramos et al. 2018). It is possible to utilize flow cytometry to identify myeloid or lymphoid antigens and so categorize acute leukemias when morphology and/or cytochemistry do not provide evidence of lineage (Bravo et al., 2014).

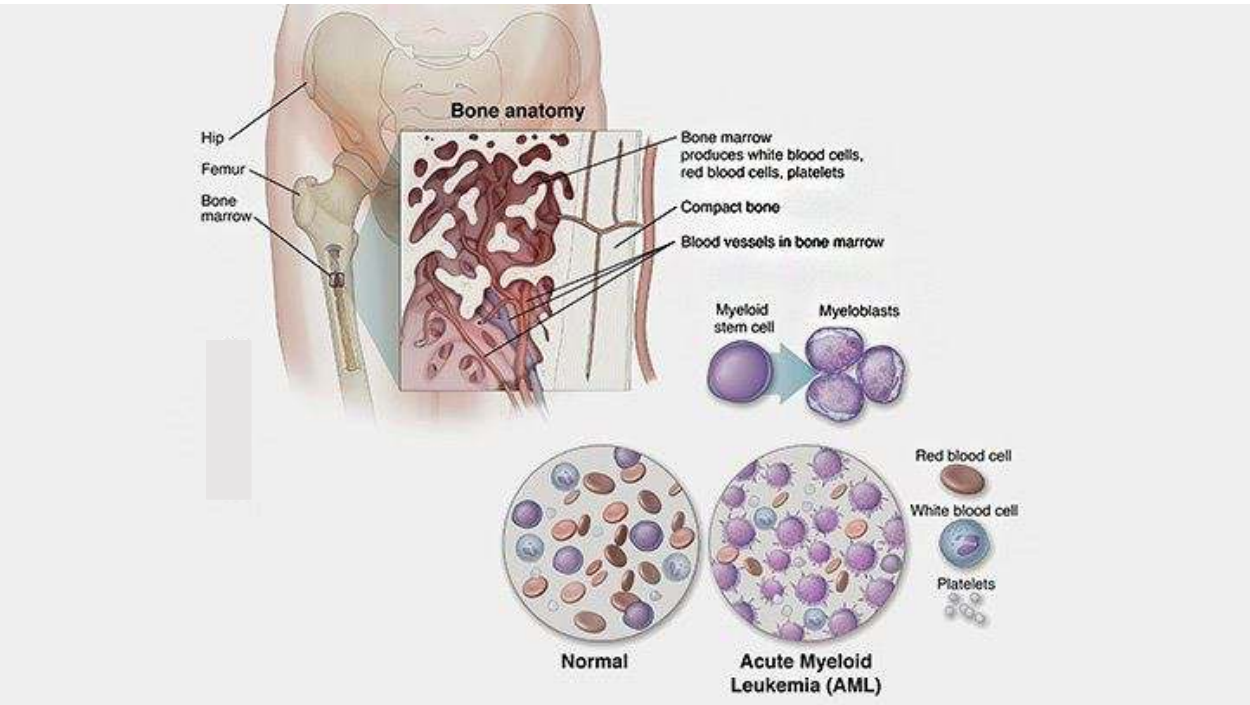

Figure 2.1: Image showing the bone anatomy of normal and acute myeloid leukemia (AML)

Source: Ramos et al. (2018)

Note: The acute myeloid leukemia (AML) that affects myeloid cells, which are the cells that produce white blood cells. As one of the principal white blood cells involved in the immune response.

Easy bruising, bleeding, shortness of breath, and an increased risk of infection are all possible symptoms (Ramos et al., 2018). There is a chance that the disease may extend to the brain, skin, or gums. AML is a quickly progressing acute leukemia that, if ignored, may be deadly within a few weeks or months (Benetatos et al., 2011).

Benzene exposure and myelodysplastic syndrome are both risk factors, as is smoking and prior chemo or radiation treatment. Red, platelet, and normal white blood cell counts fall when leukemia cells take over in the bone marrow, which is the fundamental cause. Bone marrow aspiration and particular blood tests are often used to make a diagnosis (Li et al., 2016).

Typically, chemotherapy is used as the first-line treatment for AML, with the goal of producing

remission (Bravo et al., 2014). A stem cell transplant is also an option for those who need further treatment. Depending on the cancer cells' genetic abnormalities, treatment may be tailored and the patient's prognosis can be predicted.

One million individuals were impacted with AML in 2015, and 147,000 people died as a consequence (Sun et al., 2018). It is especially frequent among the elderly. Males are more likely than females to be afflicted. More than half of persons under 60 years old and less than a third of those over 60 years old survive for five years (Sun et al., 2018). Cancer survivorship is often five to ten months in the elderly who are not well enough for intense treatment. One percent of all cancer cases and one percent of all cancer deaths in the United States may be attributed to it (Sun et al., 2018).

2.2 Epidemiology of AML

People die from acute leukemias despite the fact that they are rare (Zhou et al., 2013). Adults are most often diagnosed with acute myeloid leukemia (AML), which has the lowest survival rates of any kind of leukemia. There have been no significant improvements in survival rates for those older than 60 over the previous 20 years, despite the steady improvement in treatment outcomes (Ramos et al., 2018).

Most adult acute leukemias are acute myeloid leukemia, however in children, acute myeloid leukemias account for 15% to 20% of acute leukemias. In nations with high human development indices, it is more common. There are 2.8 and 2.2 cases per 100,000 women and men in Australia and Austria respectively, making them the countries with the highest rates (Ramos et al., 2018). As with other forms of leukemia, there is a male preponderance in AML. As one gets older, the risk increases at a faster and faster pace. Diagnosis occurs at a median age of 68 years in Western nations. As the frequency of myelodysplasia (a cancer of the blood) rises, so do the chromosomal abnormalities associated with the condition (Machova et al.,

2013). AML is diagnosed at a younger age in low- and middle-income nations, probably because the population is younger, although environmental and genetic variables may also play a role (Machova et al., 2013).

2.3 Etiology

Most instances of AML that develop in someone who was previously healthy are called de novo AML (Jhanwar, 2015). Exposure to ionizing radiation, benzene compounds, leukamogenic chemotherapeutic drugs, hereditary abnormalities and antecedent hematological illnesses are all factors that contribute to secondary AML. 4-10% of adults and children with MDS or AML may have a genetic susceptibility (Jhanwar, 2015). Bone marrow failure syndromes or inheritable germ line mutations in many genes may give rise to familial MDS/AML, however this is not always the case. Mutations in genes coding for transcription factors, including as GATA2, CEPBA, and RUNX1 among others, cause familial non-syndromic MDS/AML diseases (Santini et al., 2013). De novo AML patients often have mutations in these genes. It is possible that some of the patients are younger and there is a family clustering. Many of these individuals do not have a family history that would indicate a heritable risk; thus, a strong index of suspicion is required. Myeloproliferative neoplasms and myelodysplastic syndromes are more likely to develop into AML if a patient has this condition (Wouters and Delwel, 2016). There must be a blast count of 20% or more in bone marrow or peripheral blood or the appearance of an extramedullary buildup of blasts in order to diagnose AML. Cytogenetic and molecular mutations accumulate as the transformation progresses. Primary treatment resistance, advanced age, underlying organ dysfunction, and extended cytopenias caused by inadequate hematopoiesis all contribute to the poor prognosis associated with secondary AML, which has lower chances of full remission (Zhou et al., 2013).

2.4 Clinical presentation

In individuals with AML, the rapid onset of symptoms is due to the rapid infiltration of the bone marrow by leukemic cells and the subsequent failure of normal hematopoiesis (Zhou et al., 2013). Anemia, infection, and bleeding may occur as a result of the patient's lack of normal hematopoietic components, and these criminal leukemic cells that infiltrate the bone marrow are defective, putting the patient at danger. The most prevalent symptoms of anemia are exhaustion and exhaustion (Krishnan et al., 2022). Pallor, dyspnea, palpitations of the heart, and a general feeling of ill health have all been mentioned as symptoms of the disease. Approximately 15–20% of patients have fever, which is usually due to bacterial, fungal, or viral infection, but may also be caused by a leukemic cell load in tissues and organs (Krishnan et al., 2022). Thrombocytopenia may cause bruises, petechiae, and mucosal bleeding. gastrointestinal or genitourinary tract and central nervous system (CNS) bleeding are less frequent but more serious symptoms of reduced platelet count. Approximately 15% of adults and 5% to 20% of children with acute myeloid leukemia (AML) are found to have CNS involvement with large numbers of leukemic cells (Lauschke et al., 2019). Symptoms such as headache, blurred vision, and other neurological problems are signs that the meninges are involved (Lauschke et al., 2019). The peripheral blood contains leukemic blast cells, which are capable of invading any tissue in the body. In monocytic or myelomonocytic leukemias, extramedullary hematopoiesis is prevalent. Leukemic bone marrow's poor performance may revive organs that were active during embryonic hematopoiesis, allowing them to create cells anew. It is possible to have hepatosplenomegaly or lymphadenopathy, although it is not as common as it is in chronic leukemic patients (Milosevic and Kralovics, 2013). Gingival hyperplasia, a common sign of skin infiltration in monocytic leukemia, is a special kind of skin infiltration (Krishnan et al., 2022).

2.5 Cytogenetics

The discovery that chromosomal abnormalities play a harmful role in AML pathogenesis was made possible by the development of chromosomal banding methods (Puumala et al., 2013). In spite of the fact that the chromosomal landscape of AML is exceedingly variable, recurring chromosomal aberrations have been identified with evident pathogenic involvement in the onset of leukemogenesis (Puumala et al., 2013). Structural and numerical chromosomal abnormalities may occur at the same time. Unbalanced structural chromosomal rearrangements include deletions, isochromosomes, and unbalanced translocations, while reciprocal translocations, inversions, and insertions are considered balanced. There are a variety of numeric anomalies, such as monosomies and trisomies. As a result of the mapping of genes related with these chromosomal anomalies, various subtypes of AML have been identified, each with a unique prognosis (Puumala et al., 2013). This results in the production of aberrant chimeric fusion genes, which are involved in the transcription of transcription factors, epigenetic regulators, and nuclear pore components in the hematopoietic stem cell population. It has been revealed that these abnormal chimeric fusion genes cause defective differentiation and self-renewal of the hematopoietic stem cell/early progenitor cell, which has been proven to be an early starting event for leukaemia. Older age and secondary AML are linked to unbalanced translocations, numeric aberrations, and complicated karyotypes. One or more chromosomal abnormalities are seen in about half of individuals with acute myeloid leukemia (AML) (Krishnan et al., 2022).

2.5.1 Standard conventional metaphase karyotyping

A microscope is used to look for chromosomal structural and numerical abnormalities during a kylotyping procedure (Sadikovic et al., 2008). To isolate the chromosomes, bone marrow samples or peripheral blood may be used, as long as 10% or more of the leukemia is present

(Sadikovic et al., 2008). To raise the mitotic index, the cells are grown for 24 to 48 hours under precise circumstances. A blocking agent, colcemid, is then added to the culture media to stop the cell cycle at the metaphase stage, when the chromosomes are most condensed and visible (Sadikovic et al., 2008). This inflated condition of the cells is maintained with hypotonic solution and preservative before they are stained with giemsa stain (G-banding) and form typically evident under a microscopical pattern of light and dark banding. To identify any abnormalities, an appropriate number of cells in metaphase, ideally 20 or more, must be studied (Sadikovic et al., 2008).

Metaphase karyotyping, as it is now performed, has certain drawbacks. Detection of numerical chromosomal anomalies, low mitotic index, fuzzy unreadable chromosomes, and insufficient cells in metaphase might lead to unsatisfactory chromosomal analysis. To identify chimeric fusion genes and other abnormalities, individuals with insufficient cytogenetic analysis may use targeted fluorescence in situ hybridization (FISH) and spectral karyotyping (SKY). Metaphase karyotyping success rates vary from 73% to 98% (Shah and Rawal, 2019). The bone marrow specimen or peripheral blood sample must arrive at the laboratory of analysis within 24 to 48 hours after collection in a heparinized vacutainer. This is due to the fact that karyotyping is dependent on cells that divide spontaneously. A simple technique of cell cryopreservation prior to shipment may be carried out in nations without the ability to do cytogenetic analysis, but at the expense of a reduced yield rate of enough cells in metaphase for analysis.

2.5.2 Cytogenetic abnormalities with favorable genetic risks.

t(8:21) and t(16;16) (p13.1;q22) are known as CBF leukemias, which account for 15% of adult denovo acute myeloid leukemia cases and 25% to 30% of pediatric cases, and have a good

prognosis (Shah and Rawal, 2019). Their prognosis is comparable, but morphological and clinical discrepancies may be explained by their different secondary co-operating mutations that lead to AML. 40 percent of individuals in the excellent prognosis category will relapse. There are a total of 65, 42, and 18 percent of the individuals who have mutations in the pathways of tyrosine kinases, chromatin modifications, and the cohesin complex linked with t(8;21) (Krishnan et al., 2022).

A t(15;17) balanced translocation causes acute promyelocytic leukemia (q24.1;q21.2). Few occurrences of cryptic rearrangements leading to the PML-RARA fusion gene without the usual translocation have been documented (Shah and Rawal, 2019). APL kills between 15 and 30 percent of adults who are diagnosed with it, thus early detection and treatment are critical. FLT3 mutations have been found in 45% of APL patients, however their prognostic significance has yet to be established. When the FLT3-ITD/Wild-type mutational burden is more than 0.5, Schnittger et al report lower survival results (Shah and Rawal, 2019).

2.5.3 Cytogenetic abnormalities with intermediate genetic risks.

The KMT2A gene is involved in balanced translocations on chromosome 11q23. A total of 4-5 percent of adult de novo AML cases are caused by a translocation of the MLLT3-KMT2A gene (Shah and Rawal, 2019). On its own, the MLLT3-KMT2A fusion protein has little effect on leukaemia. Multiple investigations have found mutations in signaling pathways that operate in concert. An illness with such powerful genetic co-operation has a bad prognosis.

2.5.4 Cytogenetic abnormalities with adverse genetic risks.

AML patients with t(6;9)(p23;q34.1) have a distinct clinical and immunophenotypic expression, which is fewer than 1%. FLT3-ITD-associated mutations have been linked to an

increased number of co-operating mutations. It is more common in children and young adults and has a dismal prognosis (Sugimura and Ushijima, 2000).

An inversion of the 3;3 translocation (q21; Q26) occurs in less than one percent of denovo AML cases but may be present in patients with CML and myelodysplastic syndromes (Sugimura and Ushijima, 2000). A common karyotype abnormality is monosomy 7 or del (7q) and 11q23 rearrangements, both of which include cooperating mutations in signaling pathways. Genes that encode epigenetic modifiers have also been linked to myeloid transcription factors. Dysmegakaryopoiesis is expressed in a unique way in this case.

Only 2% of all new cases of acute myeloid leukemia are BCR-ABL (Sugimura and Ushijima, 2000). Those with this fusion gene are more likely to have del than those who do not have it (-7q). NPM1 mutations and genes involved in signaling pathways have been shown to have co-mutations with CBF leukemia's. Although BCR-ABL positive has been shown to be a significant risk factor in the ELN risk classification, its consequences have been found to be mostly reliant on the presence of co-occurring cytogenetic abnormalities.

The RBM15-MKL1 fusion protein is produced as a consequence of a t(1;22)(p13;q13) translocation and suppresses myeloid and megakaryocytic differentiation by activating NOTCH, among other mechanisms. This translocation is more common in newborns and young children, although it has been documented in a small number of adults as well. It occurs in less than 1% of all cases of AML.

Monosomy 5 and 7, trisomy 8, del (5q) and del (7q) are examples of numerical chromosomal anomalies (Thomas et al., 2016). Trisomy 8 is the most frequent number anomaly, appearing in 10 to 15 percent of individuals either on its alone or in combination with other clonal chromosomal disorders (Thomas et al., 2016). In 10-20% of AML patients, a complex karyotype will be found. In order to have a complex karyotype, you must have three or more

chromosomal abnormalities that are unrelated to one another. This definition excludes chromosomal abnormalities seen in people with low or intermediate genetic risk. It is possible that some people have two or more autosomal monosomies or one monosomy with a structural chromosomal defect, which excludes CBF-AML from the monosomal karyotype definition. Older age, treatment, and AML linked to myelodysplasia all increase the risk of numerical and imbalanced translocations. Chromosomal abnormalities such -17/abn (17p) and TP53 deficiency are also related with poor prognosis. Adults with de novo AML develop them at a rate of 5-8 percent; however, this rate rises significantly in patients who have had treatment for the disease.

2.6 Molecular Genetics

Karyotyping will not reveal any chromosomal abnormalities in 50% of individuals with acute myeloid leukemia. These individuals, on the other hand, will have varying treatment results, which indicates that their cancers are different. Mutations used to be divided into two categories before the introduction of next-generation sequencing. category II mutations affecting transcription factors contribute to poor differentiation and abnormal self-renewal of the hematopoietic stem cell in category I gene mutations that activate signaling pathways and proliferation pathways. More than 98% of cytogenetically normal AML patients have molecular alterations, thanks to the introduction of next-generation sequencing (NGS). Since these methods have allowed for a better understanding of the spectrum, frequency, different patterns of co-operation and exclusivity, and clonal development in AML, improved prognostic markers have been developed.

2.6.1 Next Generation Sequencing

Recurrently altered genes in AML may now be identified using modern molecular technologies, such as next generation sequencing, at a low cost and with an expedited turnaround time. As a result of advances in next-generation sequencing technology, it is now possible to automate the extraction of DNA from blood samples, allowing for the simultaneous sequencing of millions of DNA strands at high throughput, as well as the automated interpretation of results. It is possible to sequence all genetic mutations present in an individual's whole genome using whole genome sequencing. The entire genome of an AML patient was sequenced by Ley et al. Additional mutations that had not been documented before were discovered by him, including previously known NPM1 and FLT3-ITD mutations. NGS has been validated as a tool that can identify the driver mutations in the vast majority of AML patients by a number of other studies. Whole exome sequencing, transcriptome sequencing, and targeted sequencing are some of the other techniques used in research. Multiple genes previously characterized as being important in AML pathogenesis may now be identified using these approaches, bolstering the previously utilized prognostic model for identifying AML patients, especially in those with no cytogenetic abnormalities.

After sequencing 200 adult patients with de novo AML, the cancer genome atlas research network contributed considerably to our understanding of the molecular drivers of pathogenesis (Thomas et al., 2016).

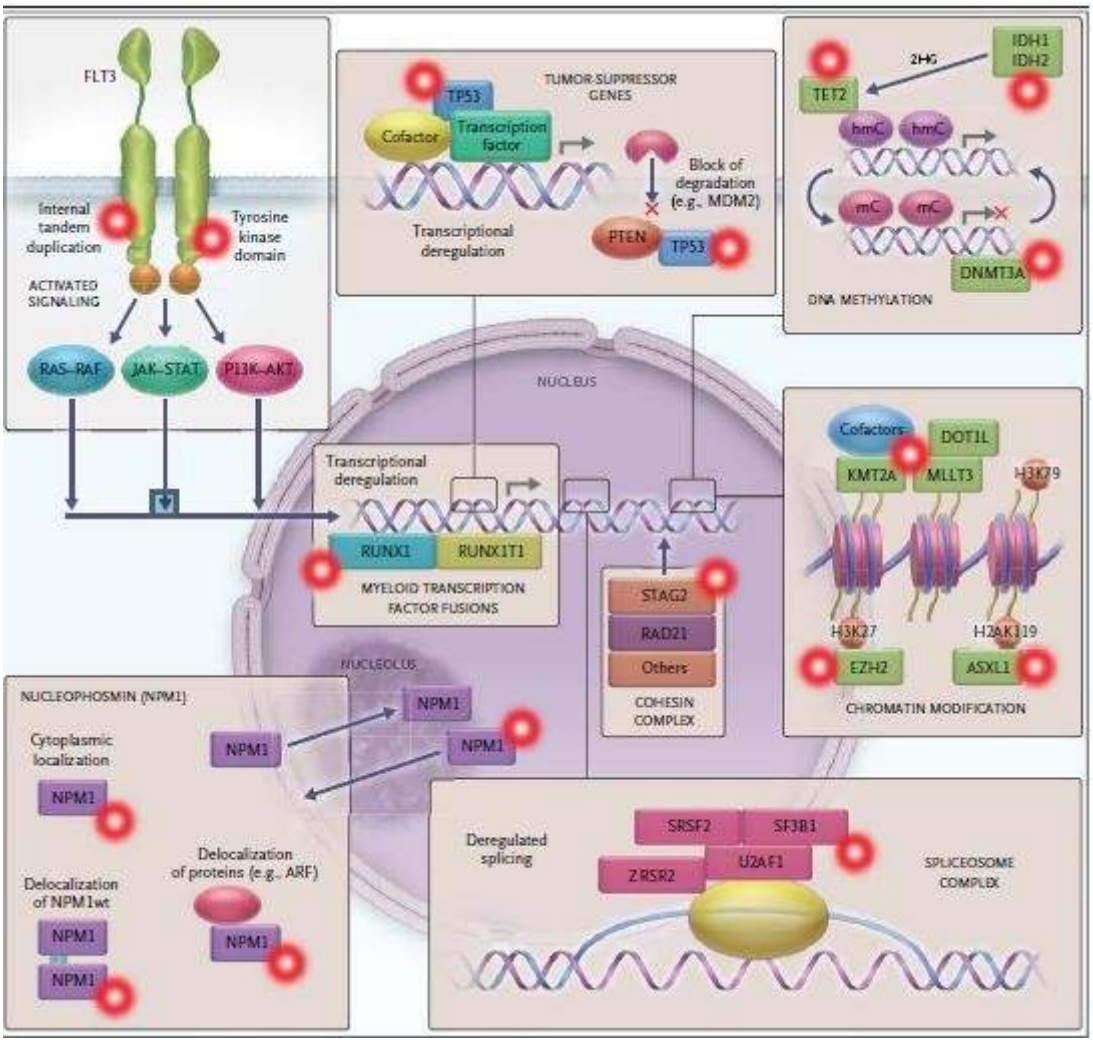

***Figure 2.3: 9 common mutated gene groups in* acute myeloid leukemia (AML)**

Source: Thomas et al. (2016)

Major gene mutations were categorized as several biological entities. DNA methylation genes (59 percent), nucleophosmin encoding genes (27 percent), chromatin modifier genes (30 percent), chromatin-modifying genes (27 percent), myeloid transcription factor genes (22%) and transcription factor fusions (18%) were the most common genetic abnormalities found in the study. Co-operation and mutual exclusivity patterns were discovered by grouping mutations into these biological categories. This database of gene mutations may be used as a baseline for future research (Thomas et al., 2016).

2.6.2 Molecular mutations with prognostic relevance

Nucleophosmin (NPM1)

Genes involved in the early phases of hemopoietic growth and expansion are upregulated when nucleophosmin (NPM1) (NPM1) is mutated, as shown by Alcalay et al. This is also the case when FLT3-ITD mutations are detected (2019). With a reported incidence of 30 percent for all patients and 50-60 percent for those with a normal karyotype, it is one of the most prevalent AML-related gene mutations (Sun et al., 2018). Patients with a normal NPM1 gene mutation and karyotype without FLT3-ITD mutations or with a modest mutational burden of FLT3-ITD constitute a group of patients with good prognostic characteristics. Co-occurrence of NPM1, FLT3, and DNMT3A may indicate a new subtype of AML.

Tumor Suppressor Genes

Proliferation and genetic changes may be prevented by tumor suppressor genes. TSG mutations affecting TP53 are the most common. 5-10 percent of newly diagnosed cases of acute myeloid leukemia (AML) are likely to be carriers of this mutation, according to the findings of Sun et al. 2018). There is a higher incidence of these mutations in AML that has been treated and in those with a complicated karyotype and aneuploidy. Poor overall survival after allogeneic transplantation may be predicted by their existence.

FMS-like receptor tyrosine kinase-3 (FLT3)

When FLT3 mutations occur, they activate proliferative signals that remain active for a long period of time. FLT3 ITDs are found in 24% of patients with cytogenetically normal AML (Baylin and Jones, 2016). A larger proportion of older individuals had these mutations, which increase the likelihood of a recurrence. If the FLT3-ITD mutation has a high mutant to wild-type allele ratio, it has a poorer prognosis than the same mutation without the allele ratio. Point mutations at codons 835 and 836 in the activating loop of the tyrosine kinase domain occur in

7% of patients, however the prognostic relevance of these mutations has not been determined. As a result, the development of AML requires collaboration with other alterations. Having FLT3 mutations together with either CBF or an 11q23 gene rearrangement portends a worse outcome. If you have the monoallelic version of FLT3, it is more likely that one will also have a CEBPA mutation. The patients in this study do not appear to be affected by it, however (Zhou et al., 2013).

Myeloid Transcription Factors

As a transcriptional factor, CCAAT/enhancer binding protein-alpha (CEBPA) helps myeloid cells mature. 10% of patients with a normal karyotype will have the biallelic CEBPA mutations and have a better prognosis than those with wild type/monoallelic mutations (Bravo et al., 2014).

When it comes to the development of all myeloid lineages, Runt related transcription factor 1 (RUNX1) is essential. Transcriptional repression and epigenetic silencing are the results of a mutation in this gene. Patients with de novo AML who have a normal or non-complex karyotype or who have AML that is linked to a family disease are more likely to have somatic RUNX mutations. T-AML and MDS/AML overlap are two more cancers that have mutations. Elderly and individuals with trisomy are more likely to have RUNX1 mutations than younger ones. If RUNX1 mutations are present in the patient's AML, the patient has a poor prognosis and a poor response to treatment (Jhanwar, 2015).

ASXL1 (Additional sex combs-like gene 1)

ASXL1 which stands for “additional sex combs-like gene 1”, is a constituent of the polycomb group of proteins having mutations happening as early events in the course of leukaemia.

Protein complexes in the polycomb group suppress gene expression via epigenetic mechanisms (Jhanwar, 2015). Even in people with a complicated karyotype, mutations within this collection of genes may lead to either an increase in progenitor cell differentiation or a decrease in cell differentiation. De novo AML patients had a 10.8 percent chance of developing them, with greater rates in the elderly and those with secondary AML (Bravo et al., 2014). AML subtypes are not exempt from their presence, since they are promiscuous and may appear in diverse places (Thomas et al., 2016).

2.7 Objectives of the study

The aim of the study is to examine the genetic and epigenetic determinants of acute myeloid leukemia (AML) pathogenesis. Specifically, this study sought to investigate the:

1 Genetic determinants of acute myeloid leukemia (AML) pathogenesis.

2 Epigenetic determinants of acute myeloid leukemia (AML) pathogenesis.

3 Link between the genetic and epigenetic determinants of acute myeloid leukemia (AML) pathogenesis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The study employed descriptive cross-sectional research in a selected hospital in the UK. The research was carried out on the adult haemato-oncology unit, which is situated on the hospital's 8th floor and accepts patients aged 13 and above.

Patients were randomly chosen until a total of 10. Therefore, the sample size was given as 10. Patients with acute myeloid leukemia were identified using morphological descriptions and/or immunophenotyping utilizing multiparametric flow cytometry or immunohistochemistry, if needed. The patients were aged 13 and above. Both verbal conviction and written consent forms were distributed to the participants stating that the purpose of the assessment was solely for academic purposes and the confidentiality of the participants would be adhered to. Patients who refused to agree or consent were not allowed to participate in the trial.

Cytogenetic abnormalities, molecular mutations, and genetic risk group were the dependent variables. Age, gender, total leucocyte count, hemoglobin level, platelet count, percent of bone marrow blasts, history of past chemotherapy or radiation, and prior hematological illness were the independent factors.

3.2 Sample Size Calculation

This research included 10 participants in all. Sample size may be calculated using the following formula using a basic random sample without replacement:

$$n = \left(\frac{z}{\quad}\right)^2 p\,1-p$$

Where,

- *m* is the margin of error (0.10 = + or – 10%);
- *p* is the estimated value for the proportion of the sample that have Acute Myeloid Leukemia (0.5)
- *z* is the z value (1.96 for 95% confidence level);

Using our factors for the population, and solving for the sample size equation, we found:

$$n = \left(\frac{1.96}{0.10}\right)^2 0.5\,(1 - 0.5) = 384$$

There were around ten patients in all that needed to be studied over the course of three months (average of 3 patients every month based on historical data available that had 150 patients over a 5-year period). This totaled to a total of N=10 individuals (population size of patients expected to be seen in the 3 month-period). The Finite Population Correction (FPC) was taken into consideration since our sample size (n=384) was much larger than the population of patients that would be accessible during the 3-month research period and fit the study requirements.

$$n' = \frac{n}{1 + \frac{n}{N}}$$

Where,

n is the sample size based on the unadjusted calculation as above, and

N is population size.

Working out the adjusted sample size *n'* using the formula above, we found a minimum sample size of 10 would be adequate for this study:

$$\dot{n} = \frac{384}{1 + \frac{384}{10}} = \mathbf{10}\ patients$$

3.3 Screening and Recruitment Flow Chart

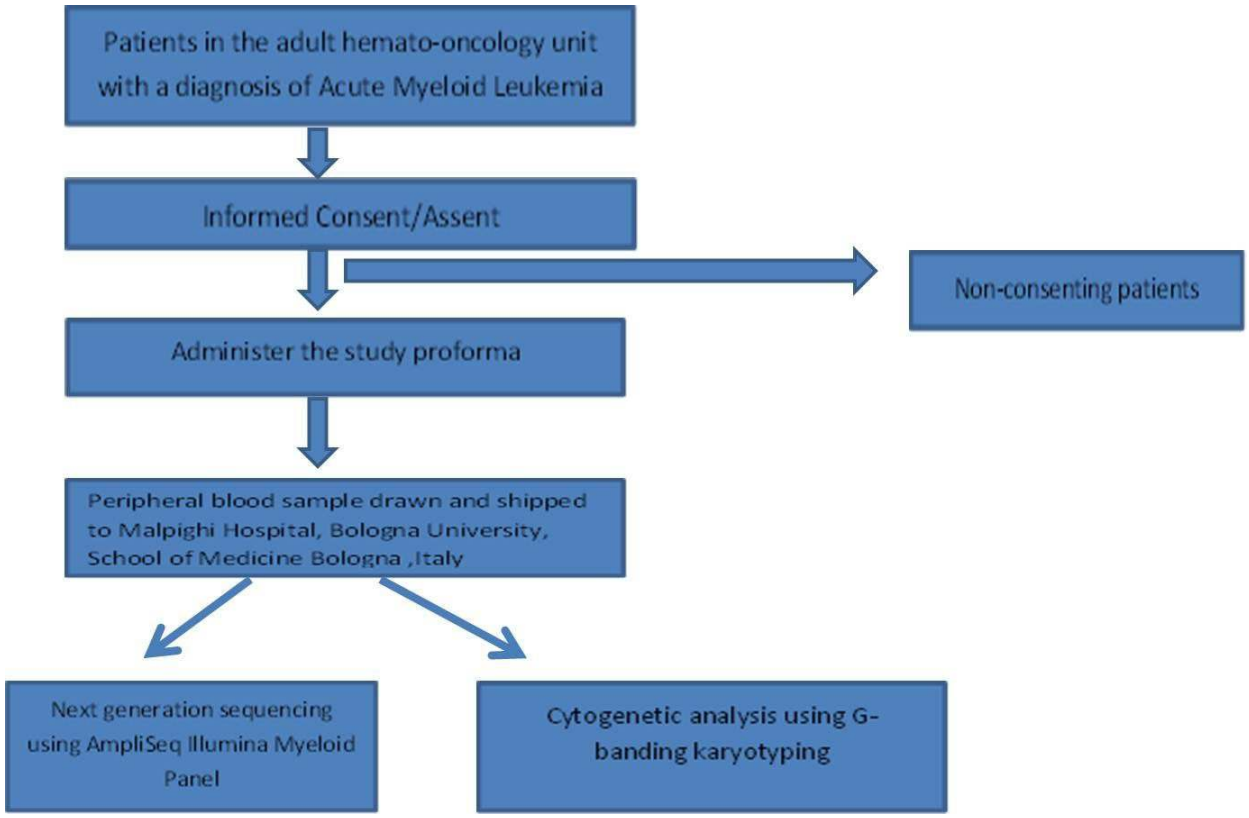

Figure 3.1 Recruitment flow chart

3.4 Data Collection

3.4.1 Clinical and Laboratory data

Patients with a morphological diagnosis of acute myeloid leukemia who were hospitalized to the adult hemato-oncology unit were informed of the trial and provided with consent/assent in English. A parent or guardian's permission or assent was acquired in the case of a minor. Clinical and laboratory data were gathered using a research proforma from participants who had given their permission or agreed to participate.

3.4.2 Laboratory Methods

3.4.2.1 Sample Collection

A standard written permission form was used to describe the process of blood collection to the patient. As soon as the patient had given their permission or approval, the cubital fossa of either arm was found and the patient was instructed to either sit or lay down in a comfortable posture. A sterile swab dipped in 10% povidine iodine was used to clean the venipuncture site. A 10cc gauge syringe fitted with an 18 gauge needle was used to extract 10cc of blood once the skin had dried. There were two separate blood draws: one for lithium-heparinized (green top) and another for EDTA-heparinized (blue top) (lavender top). Pressure was provided to the puncture site after the surgery was done to reduce bleeding and pain. The puncture wound was covered with a sterile bandage when the bleeding had ceased to prevent infection or additional bleeding. The patient was given instructions on how to properly care for the venipuncture site and how to notify a medical professional should something go wrong.

3.4.2.2 Sample Transportation and Processing

Temperature-controlled storage was used for blood samples. The sample was picked up by the scheduled courier on the same day. Blue ice packs were used to transport the blood samples in EDTA for molecular analysis. The blood samples were transported at room temperature in lithium heparinized vacutainers. The samples were brought by courier to the hematopathology division of the department of experimental diagnostics and experimental medicine. A total of four days elapsed between the time of specimen collection, the time of courier pickup, and the time of arrival to the laboratory. Additionally, the Ministry of Health approved the export of the material transfer agreement form between the two institutions.

3.4.2.3 Cytogenetic Analysis

Chromosome harvesting from whole blood: The cells were cultivated using a procedure for extracting chromosomes. As part of the study, 0.25 milliliters of fresh whole blood was taken and placed in 10 milliliters of L-glutamine (20 percent FBS, 1 percent Penicillin/Streptomycin, 1 percent Fungizone/PHA)

and incubated for 48 hours at 37 degrees Celsius. It was discovered that the final seeding densities for each sample were less than 1 106/ul below the ideal of 1-3 microliters for acceptable metaphases, prior to harvesting of cells Following the established process, samples were collected and analyzed, and none of the ten were found to have any detectable metaphases (51,52).

3.4.2.4 Molecular Analysis

Illumina next generation sequencing technologies were used to do the molecular analysis on the 10 samples. For library preparation, automated DNA/RNA extraction and amplification was used. The illumina myeloid panel was analyzed using the AmpliSeq. It is a panel of 74 genes linked to myeloid malignancies (Table 3.1) that is targeted (109). The bioinformatics technician performed the last step of data cleansing.

Table 3.1. Panel of genes tested

Hot spot genes	Full genes	Fusion driver genes	Gene Expressions
ABL1	ASXL	ABL	BAALC
BRAF	BCOR	BCL2	MECOM
CBL	CALR	BRAF	MYC
CSF3R	CEPBA	ALK	SMC1A
DNMT3A	ETV6	CCND1	WT1
FLT3	EZH2	CREBBP	
GATA2	IKZF1	EGFR	
HRAS	NF1	ETV6	
GATA2	PHF6	FGFR2	
IDH1	PRPF8	FGFR1	
IDH2	RB1	FUS	
JAK2	RUNX1	HMGA2	
KIT	SH2B3	JAK2	
K-RAS	STAG2	KMT2A	
MPL	TET2	MECOM	
MYD88	TP53	MET	
NPM1	ZRSR2	MLLTI0	
N-RAS		MYBL1	
PTPN11		MYH11	
SETBP1		NTRK3	
SRSF2		NUP214	

U2AF1 WT1		PDGFRA PDGFRB RARA RBM15 RUNX1 TCF3 TFE3	

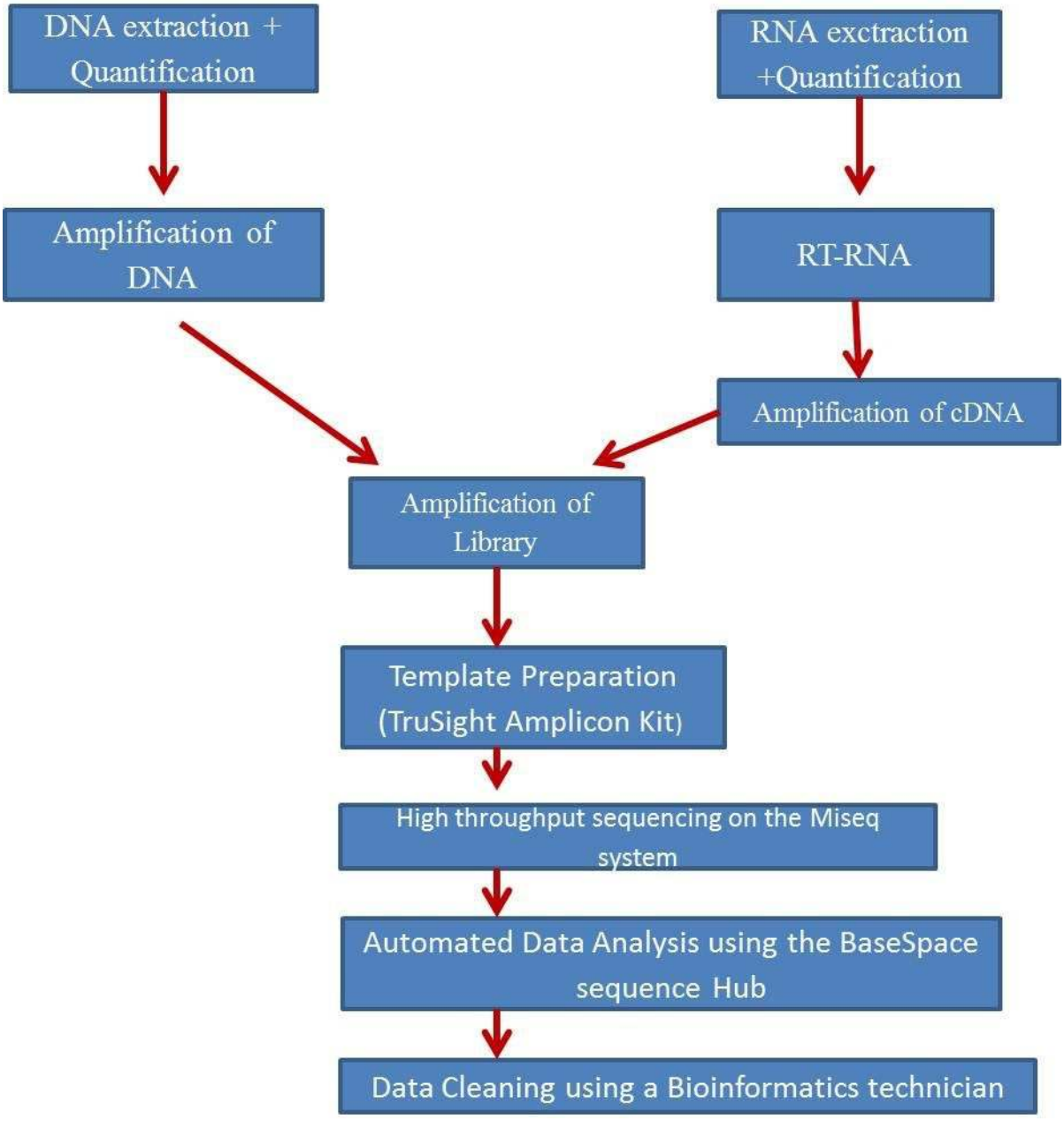

Figure 4. Work flow for Next generation sequencing

3.5 Data Management

With the support of a statistician and supervisors, the lead investigator analyzed the data. Microsoft Excel was used to input and tidy up the data. Due to the limited sample size, descriptive analysis utilizing frequencies (percentages) for categorical variables, means (standard deviations) and medians (interquartile ranges) as suitable for continuous variables was not carried out. Due to the short sample size, no formal bivariate differences were investigated. Tables were used to display descriptive statistics.

3.6 Data Protection and Study Dissemination plan

For each individual participant, the data is encoded with a unique MG code. Patients were informed of the results of the genetic testing, which were then entered into their individual medical records. The results will also be published in a peer-reviewed publication and presented at scientific conferences.

3.7 Ethical Consideration

All patients and guardians were given written informed consent or assents. It was entirely up to you whether or not you wanted to participate. A lack of permission did not compromise patient treatment. A commitment to privacy and confidentiality was evident throughout the research.

All operations were performed only after the patient or guardian signed a written informed consent form. A sterile environment was maintained at all times throughout the process. Standard standards for pain management were followed to the letter to make sure the patient was as comfortable as possible throughout and after the treatment.

CHAPTER FOUR

RESULTS

An AML diagnosis was made at the hemato-oncology unit on 15 participants throughout the research period. Each of the study's ten participants was recruited in turn. Three patients died before to enrollment, one opted out, and one began therapy before enrolment.

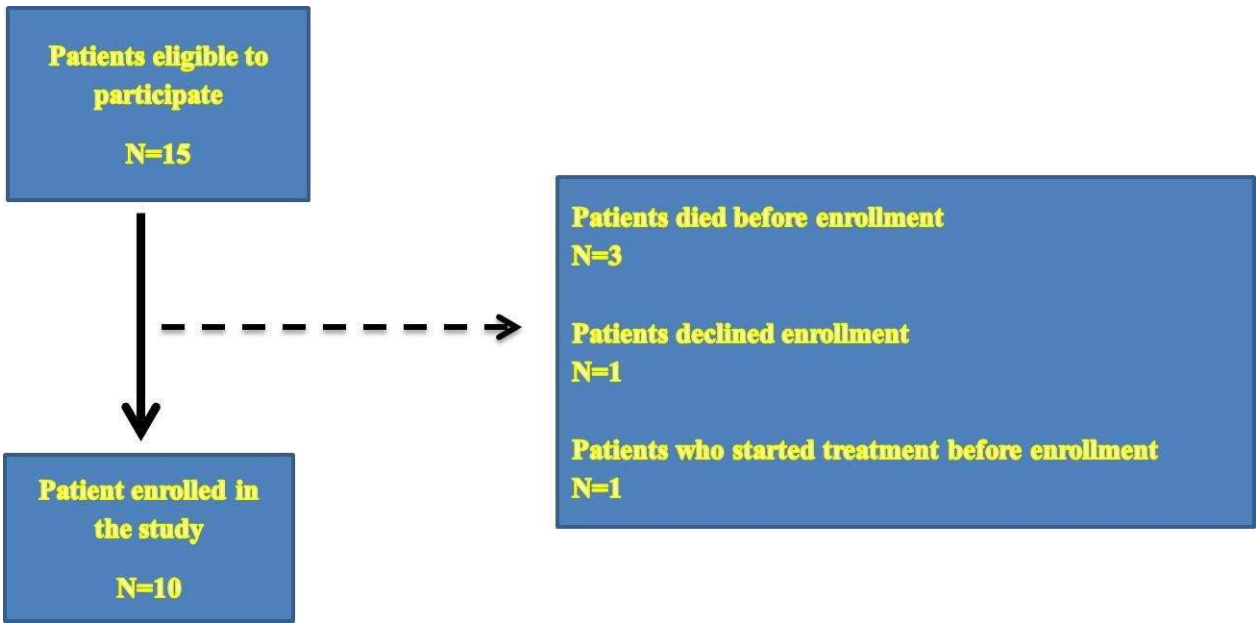

Figure 4.1. Flow chart of enrollment

4.1 Demographic, Clinical and Laboratory characteristics of the patients

Table 4.1. Patient characteristics

Case	Age Years	Gender	% of MarrowBlast	WCC /×10^9/l	HB g/dl	Platelets /×10^9/l	Prior Cytotoxic therapy or Radiotherapy	Antecedent Hematological Disorder
MG1	23	F	31	21.82	7.8	19	NONE	NONE
MG2	27	M	79	11.49	2.8	55	NONE	NONE
MG3	43	M	61	0.78	6.3	42	NONE	NONE
MG4	61	M	83	43.9	7.5	21	NONE	NONE
MG5	40	F	39	2.87	5.9	33	NONE	APLASTIC ANEMIA
MG6	13	M	45	1.99	3.9	9	NONE	NONE
MG7	70	F	76	2.80	2.9	37	NONE	NONE

MG8	**52**	**F**	**69**	**33**	**9.7**	**53**	**NONE**	**NONE**
MG9	**28**	**F**	**62**	**2.98**	**6.7**	**18**	**NONE**	**NONE**
MG10	**64**	**M**	**30**	**1.59**	**8.9**	**47**	**NONE**	**NONE**

In the research, which included ten participants, there were five men and five women between the ages of 13 and 70 years old. Table 4.1 shows that one patient had previously been diagnosed with aplastic anemia.

4.2 Molecular Mutations using Next Generation Sequencing methods

A total of 29 harmful mutations were found in 13 of the most often altered genes in AML, out of the 74 genes that were examined. One fusion gene was found, as was one frame shift, three nonsense mutations, and fourteen missense mutations. Gene mutations in two other, less prevalent AML genes were also discovered. All patients had at least one previously reported mutation, with the majority of cases having two mutations per person (Table 4.2). Three patients had WT1, TP53, STAG2, N-RAS, and mono-allelic CEBPA mutations. Two patients were found to have DNMT3A, IDH2 and PTPNII in their DNA. Each of the following mutations: PML-RARA, NF1, GATA2, FLT3-TKD, and RUNX1 was found in a patient. One patient each had ETV6 and IKZF mutations, which are uncommon in AML. Due to the insufficient sample size, pairwise mutations could not be performed to identify co-mutations.

Table 4.2. Mutational landscape according to the categories of related genes

	Mutations	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	MG9	MG10
TRANSCRIPTION FUSION GENE	PML-RARA			■							
TUMOR SUPPRESSOR GENES	NF1						■				
	TP53						■			■	■
	WT1	■							■	■	
DNA METHYLATION	DNMT3A		■		■						
	IDH2				■			■			
ACTIVATED SIGNALING GENES	FLT3-TKD				■						
	NRAS	■							■	■	
	PTPNII					■				■	
MYELOID TRANSCRIPTION FACTORS	GATA2									■	
	RUNX1					■					
	CEBPA		■	■					■		
COHESIN	STAG2						■		■		■
ETS transcription factors	ETV6						■				
IKZF1	IKZF1									■	
Total number of mutations	15	2	2	2	3	2	4	1	4	5	2

■ **Showing presence of mutation**

4.3 Description of Clinical and Molecular Abnormalities per Patient

4.3.1 : MG1

The first participant was 23 years old and had been suffering from severe dizziness, palpitations, a generalized headache and malaise, weight loss, and epistaxis for one month before presenting to the hospital. In terms of his medical background, he was not anything out of the ordinary. She is the eldest of four children, and both of his parents are small-business owners. Before starting college, he had just graduated from high school. There were no risk factors that could be established. A febrile patient who presented with a fever of 380C, blood pressure (BP) of 112/58mmHg, and pulse rate (PR) of 81 beats per minute. There was a pallor, right lower cervical lymphadenopathy, epigastria discomfort, a large splenomegaly on inspection. (WCC) of 20.92 109/l, (ANC) of 6.52 109/l, (ALC) of 5.52 109/l, (AMC) 8.33 109/l, (HB) 6.8 g/dl, and (PLT) 17 109/l were found in the total blood count (post 5 packed cells and 3 platelets). Though it was not reflective of a full examination, his initial BMA had indicated a blast transformation myeloproliferative tumor. The 210kd transcript was not detected in BCR-ABL. Some of the Auer rods in PBF had bursts of 30%. She had an overly hypercellular bone marrow that was infiltrated with immature cells that had open chromatin and conspicuous nucleoli. CD33, CD117, and MPO were all found to be positive in the IHC, whereas CD34, PAX5, TdT, CD10, and CD20 were all found to be negative. In a reactive pattern, CD 3 and CD 5 were both positive (Images not available). There was no flow cytometry performed. In addition to the normal values for uric acid (442umol), LDH (450IU), U/E/C and LFT were all within the normal range. AAFB was not found in the sputum, but candida albicans, which was not considered harmful, was. There were several lymph nodes in the hilar and mediastinum, as shown by a chest CT scan. A 26-cm-long splenomegaly, a 16.9-cm-long liver, and several para-aortic adenopathies were discovered during an abdominal ultrasound. Two NRAS (c.181C>A p.Q61K) and WT1 (c.1141 1142insCCTTGTACGGT/ c.1142 1143insCTTGTACGGTC p.A382fs) gene mutations were discovered using targeted sequencing.

4.3.2: MG2

A 27-year-old man, MG2, went to the hospital with complaints of dizziness, heat exhaustion, nocturnal cough, occipital headache, and weight loss over the last seven months. As a result of a manual evacuation she had for an incomplete abortion, she was experiencing dizziness and exhaustion. In addition, she had palpitations, bilateral edema of the feet, a prolonged cough

with no chest discomfort, and a terrible recurrent headache. Recurrent anemia necessitated the use of packed cells on many occasions. In terms of underlying medical conditions, she has none.

In addition to being a mother of two, she works as a part-time worker. She had a temperature of 380 Celsius, a blood pressure of 106/48 mmHg, and a heart rate of 133 beats per minute when she was examined. She was really pale and had both inguinal lymph nodes on both sides. (WCC) 10.59 109/l, (ANC) 7.19 109/l, (LYM) 2.35 109/l, (MON) 0.50 109/l, (HB) 3.8 g/dl and (PLT) 58 109/l were found in the total blood count. There was no flow cytometry performed. The percentage of type I and II myeloblasts detected by PBF was 73%. Marrow was found to be too cellular (FAB-M1), with 80 percent of the cells showing no signs of maturation (AML: FAB-M1). CEBPA (c.389G>T p.G130V) and DNMT3A (c.2645G>A; p. R882H) variants were discovered by targeted sequencing.

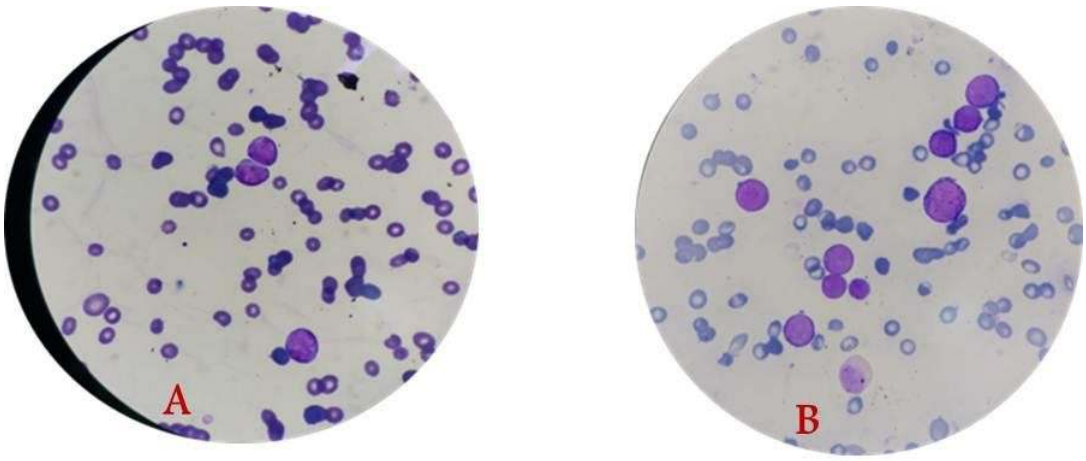

Figure 4.2. PBF and BMA images for MG2: (PBF (A) type I and II myeloblasts (B) blastswithout maturation (FAB M1).

4.3.3: MG3

MG3 is a 43-year-old man who was admitted to the hospital after complaining of epistaxis, dizziness, bleeding gums, a fever, swollen gums, fatigue, and palpitations over a period of one month prior to her admission. A tooth abscess had formed and was causing a lot of bleeding for her. After a two-week history of an upper lip ulcer with uneven edges and tenderness, she also had a history of an upper lip infection. Acute otitis media with bleeding in both ears occurred while she was in the hospital. He is one of three children in a family of four. BP was 119/77 mmHg, heart rate was 89 bpm, and body temperature was 38.50 c. The total blood count was: (WCC) 0.77 109/l, (ANC) 0.06 109/l, (ALC) 0.67 109/l, (AMC) 0.04 109/l, (HB) 7.3 g/dl,

and (PLT) 46 109/l. Because of hypocellularity, her BMA examination was inconclusive at the outset. Pancytopenia was evident in the PBF sample, with 8 percent blasts. After a second BMA, 62% of the blasts were identified as promyelocytes. There were no abnormalities in any of the tests. There was an INR of 0.97, fibrinogen of 63.7 mcg/dl (normally 169-515 mcg/l), and a d-dimer concentration of 27.04 g/ml (normally 0.00-0.50 g/ml). The PML-RARA fusion gene and the CEBPA (c.694 695insG p.V232fs) mutation were discovered using targeted sequencing.

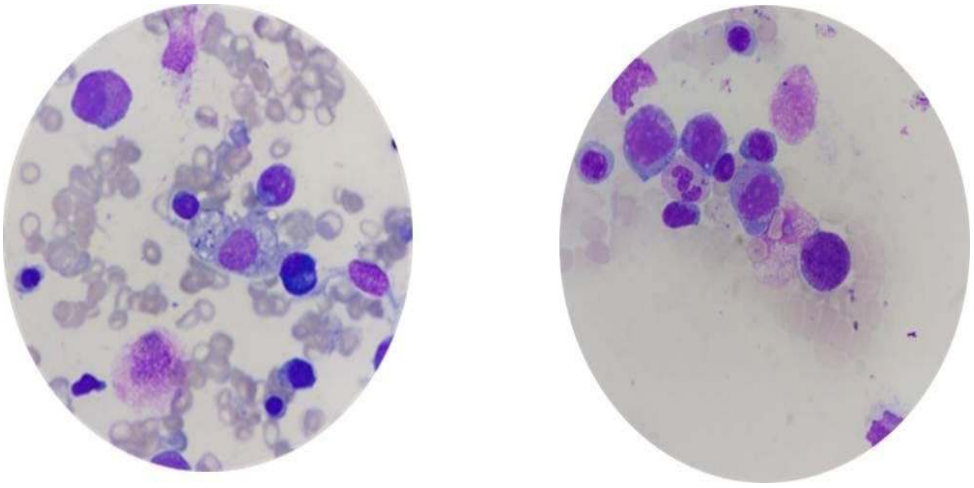

Figure 4.3: BMA Images for MG 3: Both show predominance of promyelocytes

4.2.4 MG4

For the last three months, patient MG4 had been complaining of severe per vaginal bleeding. He was 61 years old and in the midst of menopause. He also reported a month of hematuria, dysuria, and straining when peeing as additional complaints. Hypertension is her primary complication, and she is taking amlodipine and enalapril to treat it. His hypothyroidism has necessitated the use of L-thyroxine (100mcg). His asthma is well-documented, and she takes bronchodilators as necessary. Pesticides were a major source of her danger. She is a mother of three and has a husband.

Pallor and a 14-week-old pelvic mass were the key findings during the test. A large uterus with several sub-serous fibroids and a prominent cervix but no discernible tumor was discovered during a pelvic ultrasound. Squamous intraepithelial lesion was seen in her most recent pap smear, therefore she had LEEP to remove the growth. Cervical intraepithelial neoplasia was found in the findings of a biopsy (CIN). To remove her uterus and both ovaries, she was brought to the hospital. Before surgery, a total blood count indicated a WCC count of 44.8 109/l, an

ALC count (ALC) of 5.29109/l, an AMC of 33.44109/l, a Hb count (HB) of 8.2g/dL and a PLT count of 20. Approximately 82% of the nucleated cells were found to be atypical monocytoid cells with a high N:C ratio, little basophilic cytoplasm, an open chromatin pattern, and large nucleoli, as shown by PBF. BMAT had a hypercellular appearance, with blasts widely distributed throughout (fig8). CD 34, CD 117, and MPO were all detected by IHC, while PAX5 and CD3 were not. Only Amikacin and Meropenem were able to grow escherichia coli in urine M/C/S. Trace MR, moderate TR/PAH of 41mmHg, and a 62 percent LVEF were seen on the echocardiogram. There were no abnormalities in the TFTS. A normal uric acid level of 544umol/l (normal 120-430) and an LDH level of 2039 IU/L were found in the U/E/C and LFTs, respectively (normal 120-250). It was discovered that IDH2 (c.515G>A p.R172K) and DNMT3A (c.2645G>A p.R882H) both had missense mutations, which were linked to FLT3 (c.1815T>C, p.=).

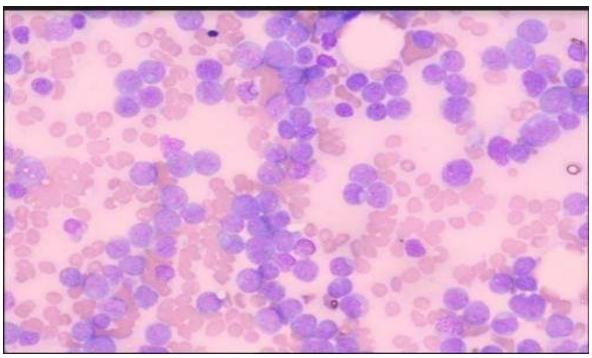

Figure 4.4: BMA image for MG4: Increased cellularity with blast infiltration

4.2.5 MG 5

Recurrent anemia has been a problem for MG 5 since 2013, when she was only 40 years old. Fecal replacement of the bone marrow was discovered in 2014, with only sparse nucleated cells and an odd megakaryocyte showing up. Aplastic anemia has been diagnosed. He was given oxymethalone 50mg TID and a slew of packed cells as a treatment. He was sent to our hemato-oncology center six years later when he developed refractory pancytopenia. Aside from dizziness and exhaustion, he also complained of palpitations, headache and epistaxis. Another risk factor was not found. With three children of his own, he made his living as a small-time dealer. During the test, the BP was 146/75 mmHg and the pulse was 86b/min. Pallor and a hemic murmur were the major things he found. There

were 2.86109/l of WCC, 0.37109/l of ANC, 2.10109/l of ALC, 5.8g/dl of hemoglobin, and 11109/l of PLT in the total blood count. Plasma explosions were infrequent, although there was significant pancytopenia in PBF. Transcription factor 5 (TdT) and CD34 were found to be positive for PAX5 and CD 117 and TdT was found to be negative for all of the other markers of marrow cellularity (images not available). 30-40% of the shots were bombs. RUNX1 (c.592G>A p. D198N) and PTPN1 (c.182A>G p. D61G) molecular mutations were discovered by targeted sequencing.

4.3.6 MG 6

14-year-old MG 6 was admitted to the hospital after complaining of nonspecific, vague stomach ache and diarrhea. He had taken over-the-counter medicine for a temporary dry cough two weeks previous to admission. Even though she had experienced the odd headache and fatigue, he did not think much of it. She had no previous medical issues to speak of. A non-smoker who had never had a drink of alcohol in his life, she had no known risk factors. He was found to be feverish, with a temperature of 38.30 degrees Fahrenheit and a pulse rate of 102 beats per minute. WCC was 2.63109/l, ANC was 0.63109/l, ALC was 1.2109/l, AMC was 5.34109/l, HB was 4.3dL, and PLT was 6109/l in the total blood count. LDH was 560 IU/L and uric acid was 234 umol/l (normal range: 120-430). (120-250). Blood cultures, CXR, and stool analysis all came up empty. PBF had a blast rate of 32%. Figure 4.5 shows that BMA had enhanced cellularity, 44% blasts, and 56% aberrant erythropoiesis series, all of which were characterized by megaloblastoid forms (FAB M6) Mutations in TP53 (c.743G>A p.R248Q), NF1 (c.7618 7619insT p.T2540fs), STAG2 (c.2359 2360insTp.A787fs), STAG2 (c.2359 2360insTT p.A787fs) and ETV6 (c.403 404insT p.A787fs) were discovered by targeted sequencing (H135fs).

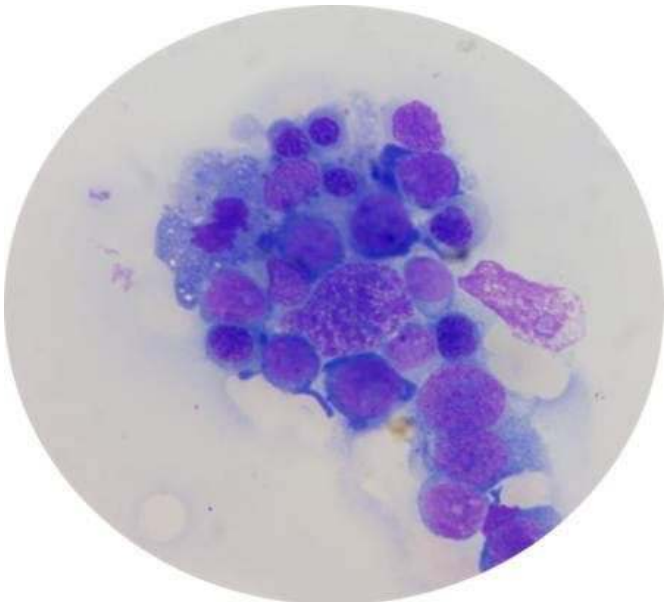

Figure 4.5: BMA image for MG 6: Abnormal erythropoiesis series exhibited by megaloblastoid forms (FAB M6)

4.2.7 Case 7: MG 7

Joint weakness, back pain, hard swallowing, non-productive cough, stomach discomfort, and chest pain were all reported by MG 7, a 70-year-old woman who was admitted to the hospital. In addition, he had two instances of syncope. For hypertension, he was taking amlodipine and enalapril in the past. She was a married father of three with no history of cancer in his immediate family. An elementary school chef in his county employed him. She was well-known for drinking and smoking three packs a year of cigarettes. A slight pallor and gingival enlargement were the primary results of his examination. (WCC) 2.6 109/l, (ANC) 0.58 109/l, (ALC) 1.80 109/l, (AMC) 0.18 109/l, (HB) 3.4 g/dl, and (PLT) 213 109/l were found in the total blood count (TBC). An AML-MRC bone marrow aspiration indicated a 75% prevalence of blasts with myelodysplasia (fig10). The creatinine level was 95 umol/l, the uric acid level was 232 umol/l, the LDH level was 137 IU/l, and the LFTS level was within the normal range. ECHO indicated an LVEF of 64%, mild MR, and moderate TR/PAH of 53mmHg on the chest X-ray. There is just one mutation in IDH2 (c.515G>A p.R172K) identified by targeted sequencing.

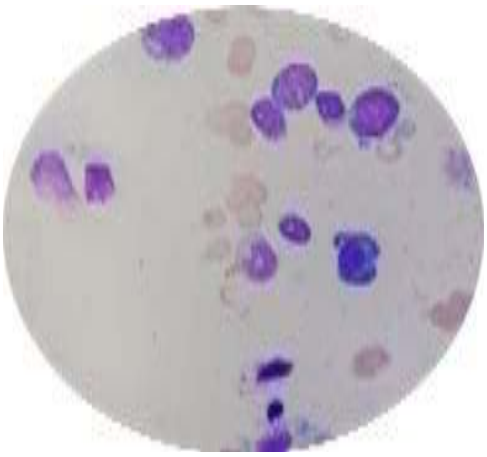

Figure 4.6: BMA image for MG 7: Blasts with monocytoid appearance and myeloblast characteristics with underlying myelodysplasia AML-MRC

4.2.8 MG 8

MG 8 was a 52-year-old female. After a 5-month history of general sickness, he was admitted to the hospital and found to be anemic. She was sent home with five packed cell units. When he returned two months later, he developed fever and anorexia in addition to the other symptoms he had previously reported. An extra five pints of packed cells and other supportive therapy were provided to him upon admission to the hospital, and we were then tasked with his care when a BMA revealed he had acute myeloid leukemia. He did not have any co-morbid conditions. She had a history of drinking, but had never smoked a cigarette in her life. Breast cancer claimed the life of her maternal aunt. As a telephone operator at a government agency, she had a husband and three children. Cervical lymph nodes that were tiny and distinct and a hepatomegaly that extended 2 cm beyond the costal edge were the key findings during the examination. To sum it up, the WCC (total blood count) was 36,6109/l, the ANC (26.6109/l), the ALC (10.3109/l) and the AMC (total blood count), as well as the hemoglobin (HB) (10.6g/dl) and platelet count (PLT) (total blood count). The percentage of explosions in PBF was 67%. Myeloblasts accounted about 70% of the BMA's cells (FAB M2). An ECHO revealed an LVEF of 72%, type I left ventricular diastolic dysfunction, and

a minor right sided pleural effusion, according to the results. X-ray results were normal. CEBPA (c.539 540insC p.Y181fs), NRAS (c.35G>A p.G12D), STAG2 (c.2359 2360insT p.A787fs) and WT1 (c.1137 1138insA p.R380fs) were found by targeted sequencing.

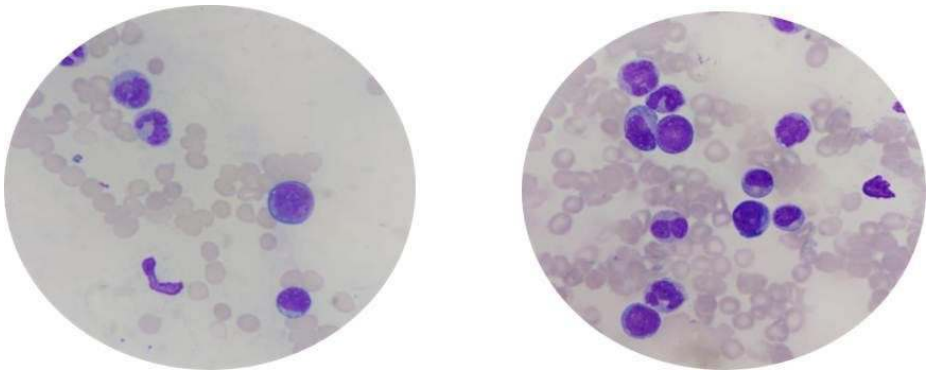

Figure 4.7: BMA image for MG 8: Hypercellular marrow with predominance of myeloblasts (FAB-M2)

4.2.9 Case 9: MG 9

It is been one month since MG 9, a 28-year-old woman, has arrived to the hospital with a variety of symptoms including a general feeling of ill health, a tendency to get tired easily, palpitations, and widespread pain. She had recently had chills, rigors, and recurring mouth ulcers, which she described as a recent occurrence. Previously, she had no known medical issues. Her mother had died when he was a baby, so his maternal grandmother and aunt raised him. Since he was a youngster, my father was blind. On examination, his temperature was 39.60 degrees Celsius, his blood pressure was 110/67 millimeters of mercury, and his heart rate was 110 beats per minute. Pallor, many mouth ulcers, and pustular tonsillitis were her most encouraging discoveries. To sum it all together, the figures are as follows: 3.49109/l (WCC), 0.90109/l (ANC), 2.04109/l (ALC), 6.9g (HB), 16109/l (PLT). The percentage of explosions in PBF was 69%. There was a notably

hyperplastic erythropoiesis in the BMA and Trephine biopsies, with the majority of erythroblasts (M6 variant-acute erythroblastic leukemia). MPO and CD 235 were both positive for CD 117 while CD34 was negative. Blasts made about 60% of the total (fig12). There were six mutations found in the TP53 gene (c.215 216insG p.V73fs), NRAS (c.35A>A p.G12D), WT1 (c.1142C>A p.S381), GATA 2 (c.953C>T p.A318V) and IKZF1 (C963/G p.Y321*).

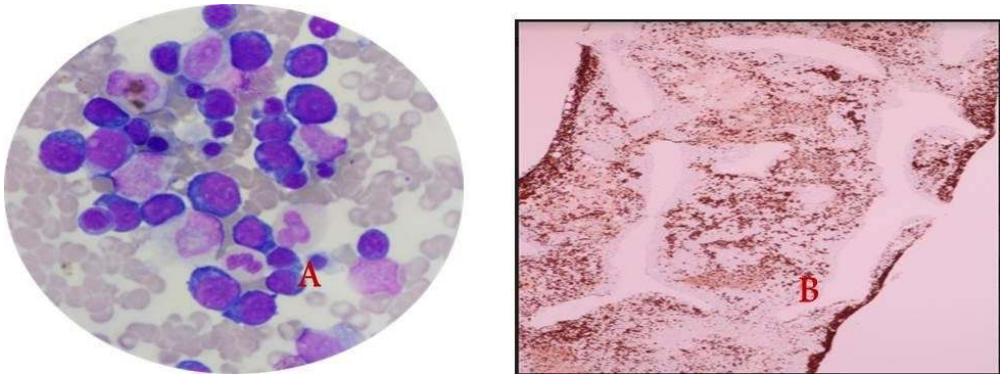

Figure 4.8: BMA and Trephine Images for MG9: (A) Marked erythroid hyperplasia, left shifted with erythroblasts seen (FAB M6) (B) CD 235 positivity)

4.2.10 MG 10

In this case, the patient, MG 10, was 64 years old and went to the hospital with a lengthy history of exhaustion easily, joint discomfort, and loss of weight. Hospitalization was necessitated by her recent deterioration in bruising and epistaxis. She did not have any known co-morbidities, and she used to be rather active for someone her age before this happened. Smoking cigarettes over a lengthy period of time has been recognized as the most significant risk factor. A mother of eight, she was a widow with a large family. Her most notable physical characteristics were her fragility and a dismal paleness. In the total blood count, (WCC) 1.59 109/l, (ANC) 0.34 109/l, (ALC) 1.19 109/l, (AMC 0.04), (HB) 7.9 g/dl, (PLT) 48 109/l were found.

Leukopenia, nRBC, and circulating blasts were detected in the PBF. According to BMA, the marrow was hypocellular with 30% blasts. Targeted next generation sequencing found TP53 (c.377A>G p. Y126C) and STAG2 (c.2245 3425insGG p. V346fs) as two of the most common genetic mutations in the general population.

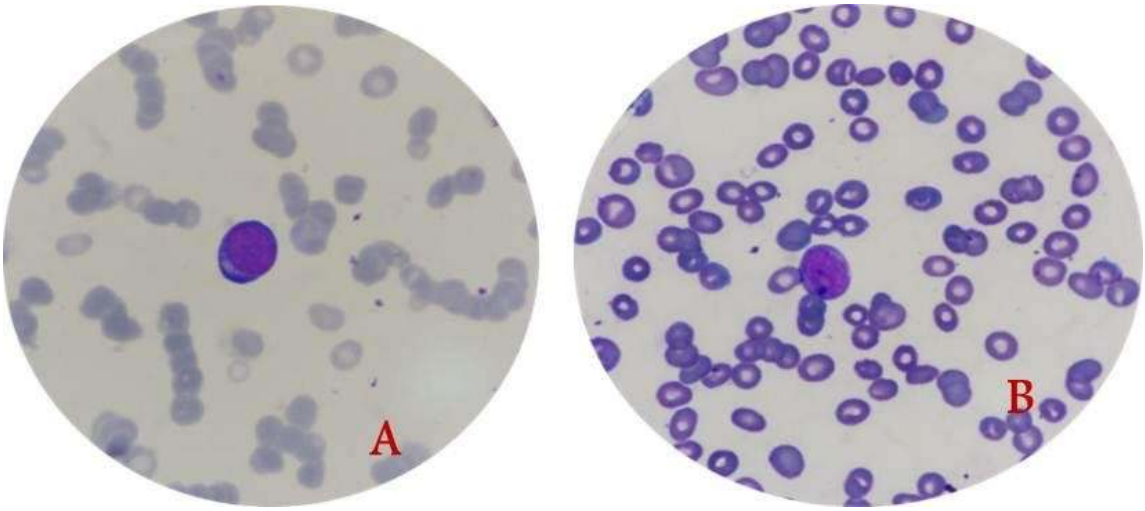

Figure 4.9: PBF and BMA images for MG10: (A) Nucleated RBC and (B)Myeloblast

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study was carried out on investigating the genetic and epigenetic determinants of acute myeloid leukemia (AML) pathogenesis. Study participants in a selected hospital in the UK hemato-oncology unit were examined for cytogenetic and molecular abnormalities. The karyotype status of the patients could not be reported since no peripheral blood cells were cultured. A longer journey to the lab was the most probable culprit of this miscalculation. Researchers found that individuals with AML in in the selected hospital in the UK had harmful mutations that give each patient a distinct genomic profile and contribute to the wide range of disease outcomes seen among patients in the population as a whole. As a consequence, we cannot make any solid conclusions about the prognostic significance of these mutations due to a lack of cytogenetic investigation and the following outcomes. Using next generation sequencing in the diagnosis of our AML patients will enable us to fine-tune and tailor the treatment of patients with AML coming to hospitals in the UK in the near future.

5.2 Limitations of the Study

This result cannot be extended to other patients with acute myeloid leukemia in the UK since the study cohort is small and restricted to a single hospital.

In order to make meaningful comparisons with other research, the study was limited by the small sample size since the researcher could not ascertain the co-mutation patterns of genetic lesions and their frequency.

As a consequence of the low mitotic index, the researcher unable to determine the prognostic significance of the identified mutations in cytogenetic analysis.

5.3 Recommendations

1. A cytogenetic laboratory should be established in our nation so that karyotype anomalies may be quickly and affordably examined.
2. As cytogenetic metaphase investigations are cumbersome and time-consuming, we may benefit from newer sequencing methods that incorporate karyotyping with mutational screening.
3. Genetic abnormalities linked to AML patients in the selected hospital in the UK need to be studied in more depth in the future.
4. This material is excellent for cytogenetic research in our setup, which requires a longer transit time to a laboratory specializing in this field.

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