الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria

وزارة التعليم العالي و البحث العلمي
Ministry of Higher Education and Scientific Research

Mentouri Constantine Brothers University
Faculty of Natural and Life Sciences

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Department of Animal Biology

Thesis presented for the graduation of a Master of Science
Field: Natural and Life Sciences
Sector: Biological Sciences
Specialty: *Genetic*

Title:

Contribution to the genetic study of acute leukemia in Eastern Algeria

Presented and supported by: ATTAF Sonia **09/26/2021**
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Academic year
2020/2021

Acknowledgment:

First of all, we want to thank GOD for the strength we had to do this work.

. We would like to express our sincere thanks for our supervisor **Doctor AYACHI Sarayah** for her patience with us, her leadership spirit, her competence and her advices which were really very helpful for our work. Thank you Madam.

We also want to express our gratitude to our co-supervisor, **Dr. Rezgoune Med Larbi,** for his guidance and presence with us during our university experience

Our jury president **Prof. Chellat djalila.** You do us the honor of accepting the presidency of the jury for this thesis. We ask you to accept our respectful considerations.

Our examiner **Dr Semmam Ouerda**, You do us the honor of taking part in the jury by accepting to examine our thesis, we hereby express our sincere gratitude to you.

DEDICATION

My parents:
Thank you for your unconditional support about my studies.
I am honored to have you as parents. Thank you for giving me a chance to prove and improve myself through all my walks of life.
I love you.
My brothers 'Hamid, Mouad and Wassim'.
For whom I wish a bright future.
My sister:
I deeply love and wish you every success in your studies and your life.
My family:
Young and old, near or far, To all those who bear the tow name: Attaf and Sifour.
My partner in crime and studies Meissoune.
Thank you for believing in me; for all the memory we shared together.
Finally, I thank all those who assisted, ncouraged and supported me during this studies.

SONIA.

Dedication

There are no enough words to thank both of my parents, the two people were and will always be with me, thank you, mother for your unconditional love and sacrifices, thank you, father for making me always feel that I'm the best person alive, for being always proud of me and there for me no matter what. So I dedicate this work to them.

To my brother Seif Eddin and my little beloved brother Wassim. And every single person in my both families "Benmansour" and "Bounefikha" who I know they are happy more than me in this moment.

To my uncle "Bounefikha Issam " for being my garden angel.

To my best friend for a life time "Boudemagh Imene" for all your support, for all the unforgettable moments that we made in this university and out of it.

A special thanks to my classmates for the good and bad moments that we go through together.

And finally, I want to thank myself for not giving up and for achieving this moment where I make myself proud of me.

Thank you.

Nihel

Dedication :

In memory of my father who encouraged me to enroll in a master's degree and left me before this job does not see the light of day.

I dedicate this work to:

My dear mom who supported me on the last day.

My sisters 'Rahil and Nardjess'.

All my friends especially 'Sofiane and Ikram'.

My big family especially "Lina and Doua".

"Sonia," my partner and right-hand the person who has accompanied me throughout my studies,

Before I finish I owe the success of this work to Ms. "Sarayah Ayachi".

And last but not least, I wanna thank me, for believing in me, for doing this hard work, for never quitting and I wanna thank me just for being me all the time.

Meissoune.

Abbreviation:

ALL: Acute Lymphocytic Leukemia

ALM: Acute Myeloid Leukemia

AHD: Antecedent Hematologic Disorder

ACS: American Cancer Society

APL: Acute Promyelocytic Leukemia

ATRA: All-Trans Retinoic Acid

AF: Atrial Fibrillation

BM: Bone Marrow

BMT: Bone Marrow Transplantation

BCR: Breakpoint Cluster Region

CD: Cluster Of Differentiation

CLPs: Common Lymphoid Progenitors

CALLA: Common Acute Lymphocytic Leukemia Antigen

CLL: Chronic Lymphocytic Leukemia

CML: Chronic Myeloid Leukemia

CMML: Chronic Myelomonocytic Leukemia

CALM: Calmodulin.

Cig: Cytoplasm Immunoglobulin

CMPs: Common Myeloid Progenitors

CBL: Casitas B-Cell Lymphoma

CBF: Core Binding Factor

CHU: Centre Hospitalier Universitaire

C-Kit: V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog

CRBT: Biotechnology Research Center

EFS: Event-Free Survival

ETV6-RUNX1: ETS (E Twenty Six) Variant Gene 6

FAB: French-American- British

FCI: Flow Cytometric Immunophenotyping

FISH: Fluorescence In Situ Hybridization

FLT3: Fms Related Receptor Tyrosine Kinase 3

GWAS: genome-wide analysis

GTP: Guanosine-5'-Triphosphate.

HRAS: Harvey Rat Sarcoma

HSC: Hematopoietic Stem Cell

JAK2: Janus Kinase 2

K-RAS: KAY-Ras.

MYH11: Myosin Heavy Chain 11

M4Eo: Acute Myelomonocytic Leukemia With Abnormal Eosinophils.

MDS: Myelodysplastic Syndromes

MLL: Mixed Lineage Leukemia

N-RAS: Neuroblastoma Ras

NK: Natural Killer Cells

POG: Pediatric Oncology Group

PCR: Polymerase Chain Reaction

PTPN11: Protein Tyrosine Standard Phosphatase Non Récepteur 11

PML: Promyelocytic Leukemia Protein

RBC: Red Blood Cells

SMD: Senile Macular Degeneration

SmIg: Superficial Membrane

SVM: Support Vector Machines

SNP: Single-Nucleotide Polymorphism

SEER: Surveillance, Epidemiology And End Results

TK: tyrosine kinase

WHO: World Health Organization

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INTRODUCTION

Introduction

Cancer is one of the most frequent diseases that burden the human health worldwide, there are no less than 200 types grouped into four groups; liquid cancers, solid cancers, metastatic cancers and secondary cancers [1].

Leukemia is a cancer of the early blood-forming cells that effect hematopoietic cells. It is believed to arise through a multistep accumulation of genetic changes that result in deregulation of cell growth, differentiation, and programmed cell death. Leukemia can be classified according to the speed of development of the tumor into two main groups: chronic leukemia and acute leukemia. In this study, we will work on acute leukemia with both its subtypes [2].

A cytomorphological and immunophenotypic classification are the necessary tools to identify cell lineage and have a precise diagnosis of acute leukemia but sometimes they are not enough to determine all the subtypes, that is where cytogenetic and molecular variatiations investigation are needed.

in many instances, the cytogenetic results at diagnosis provide the most single important parameter for determining prognosis so far. Numerous recurrent karyotype abnormalities have been described in acute leukemia. These findings on the chromosomal level were followed and supplied by molecular studies that have identified genes involved in leukemogenesis. Even more, molecular markers were found to characterize specific subtypes of acute myeloid and lymphoid leukemia and completed the genetic marker profile.

The identification of specific chromosomal abnormalities or molecular markers and their correlation with cytomorphological features, immunophenotype as well as clinical outcome led to a new understanding of this disease as a heterogeneous group of distinct biological entities. The importance of cytogenetic and molecular genetic findings for classification and for the understanding of pathogenetic mechanisms is increasingly appreciated in clinical context and was translated also into the new world health organization classification of hematologic malignancies that uses cytogenetic abnormalities as a major criterion.

The aim of this study will be:

- To conduct a literature review and gather information on these hemato-cancerous pathologies, while focusing on the genetic process involved in their occurrence.
- A study of the data provided in all acute leukemia's patients files.
- To describe the biological and clinical characteristic of acute leukemia patients, with a focus on the essential diagnosis tools.
- Investigate molecular variations in two genes known to be the most common mutations in acute leukemia.

Bibliographic Part

Chapter 01:
Hematopoetic System

1 Cellular blood components

Blood is a lifesaving liquid organ; it is a mixture of cellular elements which are: red blood cells (erythrocytes), thrombocytes, lymphocytes, and phagocytic cells. Lymphocytes and phagocytic cells together compose the white blood cells (leukocytes). Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug in a damaged blood vessel to prevent blood loss [3].

1.1 Red blood cells

Red blood cells (RBC), also known as erythrocytes. Considered exclusively as transporters of oxygen and nutrients to the tissues, more recent experimental evidence indicates that they are important interorgan communication systems with additional functions, including participation in control of systemic nitric oxide metabolism, blood rheology, and viscosity [4].

The mature erythrocyte lacks a nucleus and some organelles. This allows having more room to store hemoglobin, enabling the cell to transport more oxygen. RBCs are also biconcave in shape; this shape increases their surface area for the diffusion of oxygen across their surfaces.

1.2 White blood cell

White blood cells or leukocytes are capable of motility and are part of the immune system; however, the specific roles and functions vary for each blood cell type. They circulate in the blood and mount inflammatory and cellular responses to injury or pathogens. An abnormal rise in the number of white cells is known as leucocytosis, whereas an abnormal reduction is called leucopenia [5].

Leukocytes can be classified as granulocytes and agranulocytes based on the presence and absence of microscopic granules.

1.2.1 Granulocytes

Granulocytes can be distinguished from one another by the morphology of their nucleus and their size, they also have azurophilic granules (lysosomes) and specific granules that contain substances unique to each cell's function [6].

1.2.1.1 Neutrophils

Have an uneven form and several lobes in their nucleus. When activated, they migrate into the tissues via diapedesis. When activated in connective tissue, they undergo apoptosis and are then removed by macrophages. Neutrophils account for around 50% to 80% of white blood cells.

1.2.1.2 Eosinophils

Have a bi-lobed nucleus with large cytoplasmic specific granules that are eosinophilic. They are phagocytic cells that may migrate into various tissues of the body and participate in defense processes, hypersensitivity and inflammation. They represent less than 3% of leukocytes.

1.2.1.3 basophils

Are the granulocytes with the smallest number of granules. These granules include histamine and heparin, which are produced in response to inflammation and tissue damage, but they are not phagocytic in nature. Basophilia improve blood flow to the wounded tissues and release substances that attract other leukocytes to this area.

1.2.2 Agranulocytes

1.2.2.1 Monocytes

Are precursor cells for the mononuclear phagocytic system, which include cells such as macrophages in connective tissue and organs. These cells are scavengers that engulf intact or dead microbes and then mop up cellular debris from areas of infection.

1.2.2.2 Lymphocytes

Constitute approximately 25% white blood cells, are of varying sizes, and have spherical nuclei. Lymphocytes subdivide into several groups using the cluster of differentiation (CD) markers. The major groups are B lymphocytes and T lymphocytes.

The B cells that develop in the bone marrow and the T cells that grow in the thymus gland are the two basic types of cells. Once B-cells and T-cells are activated, the response takes two different forms. B cells, also known as plasma cells, that have been activated produce highly specific antibodies that bind to the antigen that elicited the immune response. Helper T cells produce substances that attract other immune cells and aid in the coordination of their onslaught. Cytotoxic T cells, on the other hand, assault virally infected cells [7].

1.3 **Platelets**

Are irregularly shaped fragments of cells that circulate in the blood until they are either activated to form a blood clot or are removed by the spleen. Thrombocytopenia is a condition of low levels of platelets and carries an increased risk of bleeding [7].

2 Bone marrow

Bone marrow (BM) is a soft, gelatinous, and spongy tissue that fills the cancellous bone cavity. And consists of hematopoietic islands, adipocytes, stromal cells, and vascular tissue (endothelial cells and smooth muscle cells) (Figure 1).

Between hemopoieticically active (red) and inactive (yellow) marrow, the relative proportions of these elements differ. At the time of age, red marrow in the bone mass shifts from the core parts (spine, ribs, and upper and lower limbs) to the extremities (e.g., elbows, wrists, knees, ankles, and toes). This is replaced by yellow marrow, which is found in appendicular (armpits, buttocks, thighs, etc.) bones [8].

All blood cells in humans are formed in the red bone marrow, with the exception of lymphocytes, which are created in the marrow and mature in the lymphoid organs. Along with the liver and spleen, red bone marrow plays a role in the elimination of old red blood cells. Yellow bone marrow is usually used as a fat storage facility, although it may be changed to red marrow in specific circumstances, such as significant blood loss or fever [9].

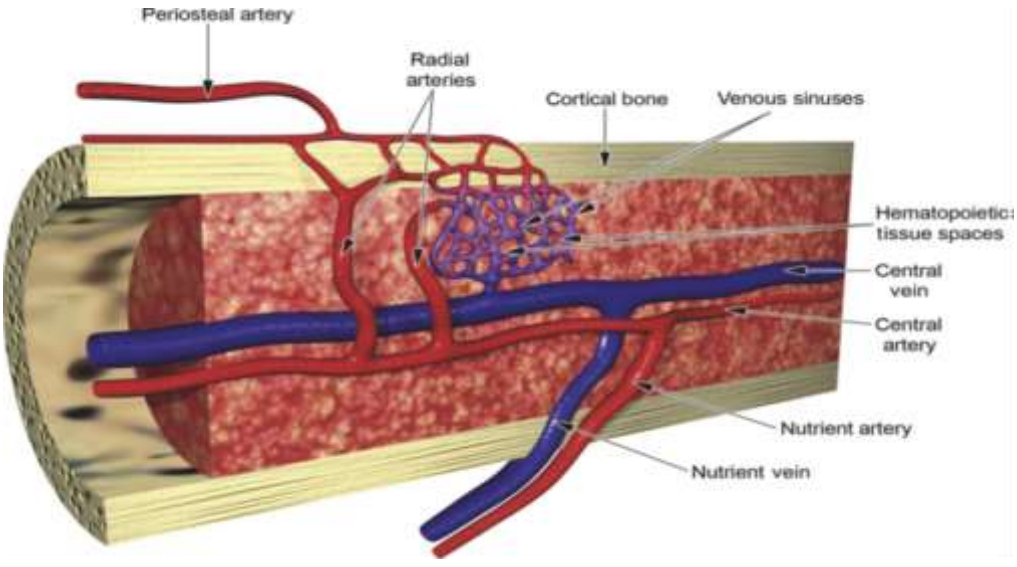

Figure 1: Schematic illustration of the bone morrow

3 Hematopoiesis

Haematopoiesis is a normal development of different types of blood cells in order to satisfy daily requirements and to adapt to increased demand, such as damage or illness [10]. The primary locations of hematopoiesis change throughout life. At the beginning of the fetal period, it begins in the yolk sac, eventually transitioning into liver, spleen, and finally the bone marrow and lymph nodes. It is maintained in these final locations for the duration of adult life except in pathological cases where it can return to its former sites.

All blood cell types arise from pluripotent stem cells in the bone marrow with the ability to re-new themselves. Hematopoietic stem cell (HSC) can promote the creation of more stem cells or differentiate them into two distinct groups of progenitor cells: common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). Lymphoid lineage cells comprise T, B, and natural killer (NK) cells, while myeloid lines comprise megacaryocytes, erythrocytes, granulocytes, and macrophages (GM). Under normal physiological conditions, the progeny of CLP and CMP cannot be overlapped **(Figure 2)** [8].

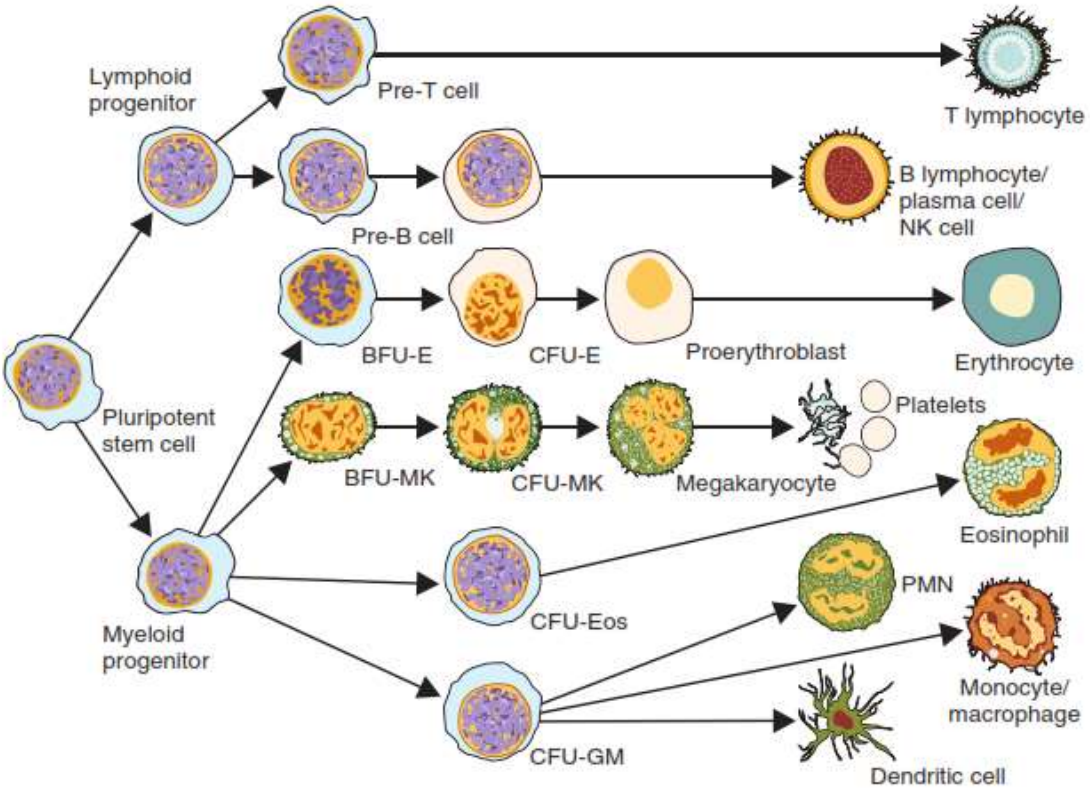

Figure 2: Overview of Hematopoiesis

Overview of Hematopoiesis. Hematopoietic lineages are outlined in this simplified overview of hematopoiesis. BFU-E, Burst-forming unit–erythroid; BFU-MK, burst-forming unit–

megakaryocyte;CFU-E, colony-forming unit–erythroid; CFU-Eos, colony-forming unit–eosinophil; CFU-MK, colony-forming unit–megakaryocyte; CFU-GM, colony-forming unit–granulocyte–macrophage; NK, natural killer; PMN, polymorphonuclear leukocyte [11].

3.1 **Hematopoietic stem cells**

In one perspective, stem cells are "primal undifferentiated cells that maintain the capacity to develop into other types of cells." This permits them, as long as the organism is alive, to operate as a repair mechanism for other cells to replenish themselves [12]. Stem cells are defined by two important properties that enable them to be separated from other cells:

3.1.1 Self-renewal

Is the process by which a stem cell divides asymmetrically or symmetrically to generate one or two daughter stem cells that have a developmental potential similar to the mother cell [13].

3.1.2 The capacity for differentiation

Under certain physiological or experimental conditions, stem cells are able to differentiate into specialized cells [14].

Throughout life, hematopoietic stem cells (HSCs) give birth to all blood cells in the body. They may renovate their own population, maintaining long-term capacity for blood regeneration and distinguishing themselves from all blood lines. Blood is different from other cells in that it has a high turnover rate: 1x106 blood cells are produced every second in a human body [15].

Chapter 02:
Leukemia

1 Definition

The word leukemia represents the general description of blood cancers. It is a clonal disease characterized by increased, uncontrolled abnormal hematopoietic cells and the accumulation of leukemia immature cells known as blasts that lose their ability to differentiate in the bone morrow and the blood stream.

They can be classified as either acute or chronic according to the degree of cell differentiation and as myelocytic or lymphocytic according to the predominant type of cell involved.

Acute leukemias are characterized by abnormal cells that are less mature, develop quickly and leave the bone marrow as dysfunctional blasts. These blasts crowd out healthy cells in the bone marrow, causing the rapid onset of symptoms [16].

2 Pathophysiology

Leukemia occurs due to malignant transformation of pluripotent hematopoietic stem cells. Rarely, it can also involve a more committed stem cell that has a limited self-renewal capacity. In acute leukemias, these malignant cells are generally immature, poorly differentiated, abnormal blasts that can either be lymphoblasts or myeloblasts. These blasts can undergo clonal expansion and proliferation, leading to replacement and interference of the development and the function of normal blood products with malignant cells, leading to clinical symptoms.

In ALL, chromosomal translocation or abnormal chromosome numbers can lead to mutations in precursor lymphoid cells leading to lymphoblasts. Common mutations include t(12;21) and t(9;22), also known as the Philadelphia chromosome. In AML, chromosomal translocations, rearrangements, and gain or loss of chromosomes can lead to mutations and abnormal production of myeloblasts. One important translocation is t(15;17), which leads to the fusion of retinoic acid receptor alpha (RARA) and a promyelocytic leukemia transcription factor (PML). This leads to the development of acute promyelocytic leukemia (M3) [17] [18].

3 Classification

3.1 **Classification of acute myeloid leukemia (AML)**

AML is characterized by the hyperplasia of blasts, but in this case, of myeloid origin. It accounts for half of the leukemia cases diagnosed in teenagers and people in their 20s. It is the most common acute leukemia in adults.

There are two most commonly used classification systems for AML, the most widely used of which is the french-american-british (FAB). However, the most recently established system is that of the world health organization (WHO).

The FAB classification system was established in 1976. According to the FAB system, acute myeloid leukaemia can be divided into subtypes M0 through M7 based on morphological and cytochemical findings (Appendix 2) [19].

The world health organization classification of AML classification system was based on the immunological, cytogenetic, morphological and clinical findings. The revised (2008) and 2016 WHO classification system develops the FAB system and grouped acute myeloid leukaemias into four categories. (Appendix 3) [16].

3.2 **Classification of acute lymphoid leukemia (ALL)**

ALL occurs when primitive white blood cells of lymphoid origin reproduce without developing into normal B and T cells. It is the most common leukemia in pediatrics, accounting for up to 80% of cases in this group vs. 20% of cases in adults.

The FAB classification of ALL classifies the disease according to the infected cells: B, T, pre-B cells or even null, furthermore, according to the morphology of the cells, ALL are divided into three important categories: L1, L2, L3 (Appendix 4) [19].

Just like the AML, OMS classification was based on the cytogenetic aberrations. Nevertheless, with the new revision (2016), important new provisional entities with recurrent genetic abnormalities have been recognized and incorporated into the classification. These newly recognized entities are assuming increasing importance because of their association with an adverse prognosis and responses of some cases to the therapies [16].

4 Epidemiology

According to GLOBOCAN database, leukemia is the 15th most commonly diagnosed cancer and 11th leading cause of cancer death worldwide in 2018 accounting for 437,033 incident cancer cases and 309,006 cancer deaths. The disease burden is higher among males relative to females (incidence rate at 6.1 per 100,000 vs. 4.3 per 100,000 in 2018), as is the death rate (4.2 vs. 2.8 per 100,000 in males vs. Females) [20].

Globally, the total number of leukemia cases increased by 26% from 2005 to 2015, and population growth and aging accounted for all but 3% of this [21].

ALL and AML, which are important diseases in both childhood and adulthood, have bimodal age distributions. ALL is the most commonly diagnosed childhood cancer worldwide. It accounts for ~75% of leukemia cases in children of <15 yr of age, with a peak incidence rate of 7.8 per 100,000 among children 2–4 yr of age in the United States [22].

Beginning in young adulthood, the age distribution of AML incidence is exponentially shaped. SEER data show an especially sharp increase in incidence after 75 yr of age, when the rate nearly doubles that of adults aged 60–74 (209 vs. 109 cases per 1,000,000 person-years) [23].

Unlike other leukemias, which are more highly incident among Whites, the incidence of pediatric ALL is higher for Hispanics than for other racial and ethnic groups. For Hispanic children of <20 yr of age, ALL incidence was 4.3 per 100,000 in the United States from 2001 to 2014, compared to 3.4 for White and 1.9 for Black children. These rates have largely remained stable since 2008 [24].

Worldwide, AML occurs with greatest frequency in highly developed regions; age-standardized incidence is highest for both males and females in Australia (2.8 and 2.0 per 100,000), Austria (2.7 and 2.2 per 100,000), and the United Kingdom (2.7 and 2.0 per 100,000) [25].

AML incidence and mortality are higher among Whites than other racial and ethnic groups. For example, age-adjusted incidence among White males in SEER is 5.4 per 100,000, compared to 4.5 for Blacks and 4.1 for Hispanics. Even mortality rates demonstrate a similar racial pattern (3.8, 2.7, and 2.3 per 100,000 for Whites, Blacks, and Hispanics, respectively),

and SEER data indicate that these racial and ethnic trends persist across sex and age groups. [26].

In Algeria, leukemia is the 11th most common cancer with a mean of 1683 cases per a year. According to a survey report made in Algeria in 2018 that included 21 centers of hematology department, the male frequency of acute leukemia patients was higher than the female (53% *vs.* 47%) and the most common age group of AML patients was between 30 and 70 years [27].

5 Etiology

Leukemia is a multi-step process; it needs more than one factor to develop. A number of genetic and environmental factors for the development of acute leukemias have been proposed. Undoubtedly, genetics have a major role in the development of these diseases but also the effects of a variety of environmental factors, occupations and hobbies have been explored.

5.1 **Genetic susceptibility**

While for the majority of leukemia cases there are no obvious known predisposing factors, some genetic and acquired germline mutations and clonal chromosomal abnormalities are associated with increased incidence of leukemia. Somatic abnormalities are very common In acute leukemia such as aneuploidy, pseudo-ploidia, hyperploidia, TEL-AML1 translocation, MLL gene changes, chromosome deletion 5,7, Y and 9, t(8;21)(q22;q22), t(15;17) (q22;q11), trisomy 8 and other aberrations affecting chromosome 9,11 and 16, mutations occurring in the RUNX1 gene, CBFA2 and CEBPA [28].

5.2 **Congenital disorders**

Some of these disorders that usually occur in childhood like the Bloom syndrome, Down syndrome Congenital neutropenia, Fanconi anemia and Neurofibromatosis.

In addition to some micro-disorders such as polymorphisms of enzymes that metabolize carcinogens such as NAD (P) H: quinone oxidoreductase (NQO1) and glutathione S-transferase polymorphism after chemotherapy.

5.3 **Environmental exposure**

A large number of environmental causes for the development of leukemia have been suggested. These mostly involve exposure to cancer-causing agents, including chemicals, exposure to pesticides and fertilizers, construction, vocations in the oil/gas industries with exposure to benzene, infections and radiation during various stages of life. Occupations with exposure to alkylating agents and formaldehyde are also found to have a higher risk of leukemia [29] [30].

5.4 **Drug exposure**

As some cancer patients can live with their disease, their continuous exposure to radiation for treatment makes them move from other tumors to leukemia, as usually happens in cases of Bone marrow transplantation and cases of treatment of breast cancer. It is also worth noting that abnormalities in the 11q23 chromosome band after treatment with topoisomerase II inhibitors, although they are the causative factors of ALL, are more likely to lead to AML [31].

6 Diagnosis

The workup of leukemia is time-consuming. Multiple tests are needed to confirm a diagnosis, and subsequently, to stage the disease. The first step in the diagnosis of acute leukemia typically involves evaluating the peripheral blood of patients for anemia, thrombocytopenia, and leukopenia. The diagnosis of leukemia then involves testing via bone marrow aspirate and biopsy, along with peripheral blood samples that allow for flow cytometry, immunophenotyping, and genetic analysis.

Bone marrow aspirate and biopsy results often reveal a hypercellular marrow with a small number of normal hematopoietic cells and a diffuse population of blasts. Flow cytometry is a test that uses a variety of dyes and chemical substances to classify leukemia cells. Immunophenotyping is determined by the pattern of surface proteins on the leukemic cells and allows for discerning between healthy cells and leukemia cells.

Patterns of the cell surface proteins in addition to the complementary FISH and PCR techniques to analyze chromosomes help to confirm the final diagnosis and distinguish between different subtypes [32].

7 Prognosis

Long-term survival with leukemia varies tremendously based on leukemia subtype, cytogenetic and molecular findings, patient age, and comorbid conditions.

Beginning with AML, the prognosis is related to the advanced age. Since most elderly people have preexisting predisposing diseases or predisposing to leukemia and also genetic analysis where patients with t (8; 21), t (15; 17) or inversion 16 have a favorable prognosis of up to 65%, while patients with normal cytogenetic results are considered as an intermediate risk and the percentage decreases to 10% in the case of patients with low-risk cytogenetic results (especially -7, -5, or monosomy karyotype) (Appendix 5) [18].

There are also some Minor risks including +8, 11q23 and miscellaneous mutations in FLT3, TP53 and TET2 may also contribute poorly to the development of the disease and conversely some mutations help the body fight disease such as Biallelic mutations in CEBPA that are associated with longer survival and mutations in NPM1 that increase the response to chemotherapy [33].

As mentioned earlier, the age factor is a very influential factor, as patients older than 60 years have a recovery rate that is approximately half less than younger patients [34].

For ALL, initially only 20-40% of affected adults recover from the disease. Two types emerged in this regard, the first being a serious prognosis and the second less dangerous, and the indicators of the first were: no adverse cytogenetics, age less than 30 years, white blood cell count (WBC) less than 30,000/µl and complete remission within 4 weeks. The criteria for the second were adverse cytogenetics - translocations t(9;22), t(4;11) and age greater than 60 years and B-cell precursor white blood cells with a WBC count greater than 100,000/µl and complete remission is not achieved within 4 weeks.

Even though significant improvement in survival has been on the rise over the past 2 decades for the adult population, there remains a wide chasm between the overall survival of

pediatric patients compared with adults. The 5-year survival rate for people diagnosed with ALL under the age of 20 is 89%, yet this same 5-year survival milestone is only obtained by 35% of those who are 20 years of age and older. Patients diagnosed with Ph-like ALL have a very high risk of relapse and the overall survival rate is poor [35].

8 Treatment

Therapy varies significantly based on the leukemia subtype and patient factors (e.g., age, comorbid conditions). Before mentioning treatment methods, it is necessary to address the stages of treatment of this disease, which are:

8.1 **Induction therapy**

It is the first stage, which may last from four to six weeks, during which the largest possible number of cancer cells are killed in the blood to regenerate healthy cells and return to their normal function and number.

8.2 **Consolidation**

The second stage, which may extend from four to six months in the form of cycles, and this stage is carried out with the aim of killing the remaining cancer cells from the previous stage so that the cancer does not regenerate again in the body.

8.3 **Maintenance therapy**

The last and longest stage, it may last for two years with the aim of eliminating any cancer cells that have survived the first and second stages to prevent relapse.

The treatment methods

Chemotherapy

The most common form of treatment is polymorphic shake. The treatment periods are interspersed with periods of rest that the body needs to be able to recover well, and it is a method that requires at least six months where it can extend to an unknown extent.

Radiation therapy

This type of treatment relies on two methods, the first of which is to direct strong radiation of energy to a specific place in the body that contains cancer cells, and the second is to circulate the treatment to all areas of the body as part of a hematopoietic cell transplant.

Hematopoietic cell transplant

This method is based on replacing the cancerous or infected cells with new healthy cells that come from the same person before chemotherapy or from another person in order to grow and multiply to form new bone marrow to produce new blood cells in turn [36] [37] .

Chapter 03:

Cytogenetic And Molecular Genetics Of Acute Leukemia

Cytogenetic in acute leukemia

Nowadays, cytogenetic play an important role in identifying the biological basis of acute leukemia. It helped detecting acquired chromosome abnormalities that recur in AML and ALL and giving exact chromosomal location of breakpoints in leukemia-associated translocations and inversions. In most malignant cells, these aberrations occur and some of them play a Major role in the leukemogenisis while others play a role in the development of various types of acute leukemia.

The cytogenetics of acute leukemia has long been known to be of prognostic relevance, and both the number and some structural abnormalities are prognostic in both adult and pediatric acute leukemia. Here below is a list of the most recurrent abnormalities found in acute leukemia [38].

1 Recurrent Cytogenetic Abnormalities in Acute Myeloid Leukemia (appendix 6)

1.1 *Numerical* chromosomal abnormalities

Trisomy 8

It is the most frequently occurring numerical abnormality in aml. It occurs in about 5–6% of aml cases and more frequently in additional with other chromosomal abnormalities. M4 and m5 morphologic subtypes of aml are linked with trisomy 8.

Trisomy 21

It is the second most frequent trisomy and the third most common karyotypic abnormalities. It is present in about 3–6% of cytogenetically aberrant AML. However, it is rare to be found as a solo chromosomal aberration and is more often associated with other trisomies or as a secondary alteration to inv (16) or t (16; 16).

Trisomy 11

It is a nonrandom chromosomal abnormality that occurs in about 2%–3% of AML patients and 1% as isolated trisomy 11. Its association with AML's M1, M2, and M4.

Trisomy 13

It is detected in about 1–3 % of AML patients. It is frequently associated with secondary chromosomal abnormalities with t(6;9). Morphologic features in AML with trisomy 13 are heterogeneous but usually associate with AML- M0 and M1.

Trisomy 22

It is a rare but recurrent chromosomal abnormality detected in about 2–3 % of AML. Sole trisomy 22 has shown male predominance in adult AML Interestingly. Trisomy 22 is the most frequently associated secondary chromosomal abnormality in AML, with inv (16) detected in about 20 % of cases. A considerable proportion of trisomy 22 was reported to be morphologically associated with AML M4Eo.

1.2 Chromosomal Translocations

t (8;21)(q22;q22): RUNX1-RUNX1T1 Rearrangement

The t (8; 21) (q22; q22) translocation is a frequent recurrent chromosomal translocation found in 5–10% of AML patients. It corresponds with the M2 classification in certain instances of M4. t (8;21) is often found with additional numerical or structural anomalies (e.g., loss of X or Y chromosome, del(9q), +8, del(7q), −7, etc.).

t (15; 17) (q22; q21) : PML-RARA Rearrangement

Acute promyelocytic leukemia (APL) is a distinct subtype of AML. Morphologically classified M3 comprises 5–10 % of all AML cases, with an occurrence rate similar to t (8; 21) (q22; q22).

inv (16) (p13.1q22)/ t(16;16) (p13.1;q22): CBFB-MYH11 Rearrangement

It is detected in 5–8% of AML cases. The morphologic subtypes M4 with eosinophilia (AML- M4Eo), M2 and M5 are associated with the inv (16) (p13.1q22) or t (16; 16) (p13.1; q22). The vast majority are inv (16), with t (16; 16) being the least frequent. Both, however, include the CBFB gene on 16q22 and the MYH11 gene on 16p13.1, resulting in the CBFB-MYH11 fusion gene [39].

2 Recurrent Cytogenetic Abnormalities in Acute Lymphoblastic Leukemia

2.1 Numerical Chromosomal Abnormalities

High hyperdiploidy (51–65 ch)

It is one of the most common cytogenetic abnormalities observed in childhood B-ALL. It is seen in (25-30% of the patients). High hyperdiploidy is characterized by a nonrandom gain of chromosomes, including + X, +4, +6, +10, +14, +17, +18, and +21. The near-triploidiy (64-78 ch) is <1% and the near-tetraploidy (82-94 ch) is 1-2%.

Hypodiploidy

It is characterized by 46 chromosomes maximum and is seen in 5-8% of total B-ALL cases contrary to pseudodiploidy (82-92 ch), same for the pseudodiploidy wich concern the adults than the children [40].

2.2 Chromosomal Translocations

Philadelphia Chromosome - like Acute Lymphoblastic Leukemia (Ph-like ALL)

t(9;22)(q34;q11) translocation, encodes the fusion gene BCR-ABL1. This translocation is present in 3-5% of B-ALL cases and is associated with a very poor outcome. It has a prevalence of approximately 25% in a young population.

t(12;21)(p13;q22) (ETV6-RUNX1)

It is the most common recurrent translocation in B-ALL. It is estimated to occur in 25 % of pediatric B-ALL [40] [41].

MLL gene rearrangements

MLL (mixed-lineage-leukemia) gene rearrangements at 11q23 are present in 80% of all infant B-ALL cases and 10% of all adult B-ALL.

t(10;11) (p12;q14) (PICALMMLLT10, CALM-AF10

One of the most frequently detected translocations in T-ALL rearrangement resulting in fusion of the PICALM (CALM;11q14) and MLLT10 (AF10;10p12) [40] [41].

TAL1 Gene Rearrangements (1p32)

The TAL1 gene is a basic helix loop helix transcription factor, and is frequently rearranged in T-ALL. In approximately 20 % of T-ALL patients. An additional 6 % of patients have the translocations t (1; 14) (p32;q11.2) [41].

Gene alterations encountered in Acute Leukemia

Recently, much progress has been made to understand the mechanisms of leukemogenesis. ***Dick et al***, defines the existence of a hierarchical organization of leukemia, whereas. At least two gene changes with different functional consequences are necessary for the development of leukemia [42].

1 Gene mutations in Acute Myeloid Leukemia (appendix 7)

Dash et **al**. proposed in 2001 the postulate that the mechanism of leukemogenesis takes place in several stages by the accumulation of type 1 mutations, providing a proliferative advantage, and type 2, blocking the process of differentiation of myeloid cells. The two types of mutations form complementation groups, and it is rare to find two mutations of the same group [43].

Type 1 mutations provide proliferative and/or survival advantages to hematopoietic progenitors by deregulating specific signaling pathways such as those involving N-RAS and K-RAS, FLT3, and c-Kit [44]. CBL (Casitas B-cell lymphoma) [45]. PTPN11 (Protein Tyrosine standard Phosphatase Non récepteur 11) [45] JAK2 (Janus Kinase 2) [46].

Type 2 mutations concern the capacities for differentiation and apoptosis and lead, alone in the absence of type 1 mutation, to SMD-type blood disease. These mutations involve essentially chromosomal translocations. Several chromosomal translocations found in AML attend to the appearance of chimeric proteins implicated in the pathogenesis such as: CBF translocation [43]. PML/rarα **[**47]. MLL gene rearrangement [48].

1.1 Fms like tyrosine kinase (FLT3)

1.1.1 Structure:

FLT3 is a tyrosine kinase receptor, its gene is located on chromosome 13q12 [49]. It is made up of 4 separate domains: the first is the part extracellular N-terminal, formed of 5 sub-units "Immunoglobulin-like". The second part is the transmembrane domain, and then the domain juxta-membrane and finally the cytoplasmic domain, this part is composed of two tyrosine kinase domains TK1 and TK2 linked by an insert kinase (K) (Figure 3).

FLT3 plays a significant role in various regulatory processes of hematopoietic cells, phospholipid metabolism, transcription, proliferation, and apoptosis [50].

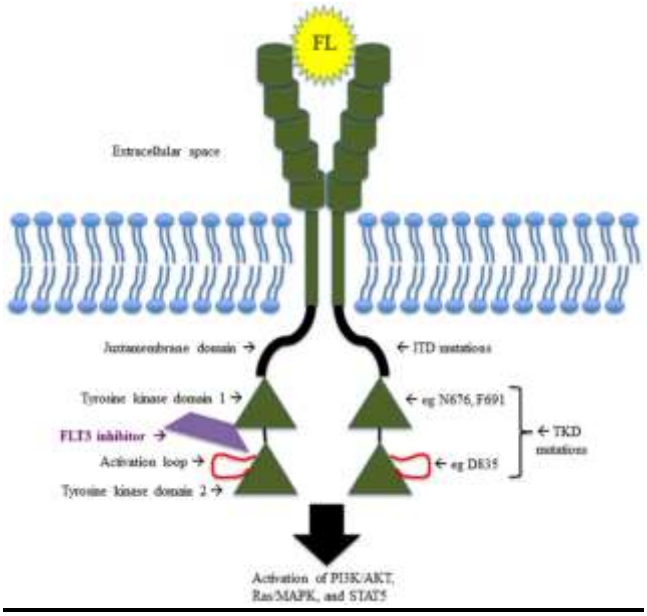

Figure 3: Schematic illustration of the FLT3 receptor [51]

1.1.2 Implication of FLT3 in cancer

The FLT3 receptor is expressed fundamentally indifferent malignant hemopathies, suggesting that it represents a significant function in the proliferation and survival of leukemia cells. In recent years, it has been proved that activating mutations in the FLT3 gene are the most frequent genetic abnormalities in LAM, and they have a significant impact on the prognosis [52].

The most common anomaly is tandem duplication (FLT3-ITD) from 3 to more than 400 amino acids located in exons 14 and 15 encodings the juxta-membrane domain, it is the most frequent, and according to the literature, it is found at a

frequency of 25-30% in adult AML patients with undetermined karyotype and 28-30% in patients with normal karyotype [53].

This alteration may induce constitutive activation, uncontrolled receptor tyrosine kinase in blasts. This activation is directly involved in the leukemogenesis. Therefore, molecules have been developed that block tyrosine kinas specifically against FLT3 with some reassuring results. AML patients with no FLT3 mutations at diagnosis can present these later during the disease, generally during relapse [54].

1.2 **KRAS (Kirsten rat sarcoma virus)**

1.2.1 Structure of KRAS

The KRAS gene (GTPase KRas) is cytogenetically located at 12p12.1. It spans near 46 kpb and has six exons; the exons 2, 3 and 4 are invariant coding exons, whereas exon 5 undergoes alternative splicing, which results in the KRASA and KRASB isoform (Figure 4). The protein KRAS is ubiquitously expressed and highly similar to those encoded by the other RAS genes (HRAS and NRAS); all of them are known as Ras superfamily GTPases.

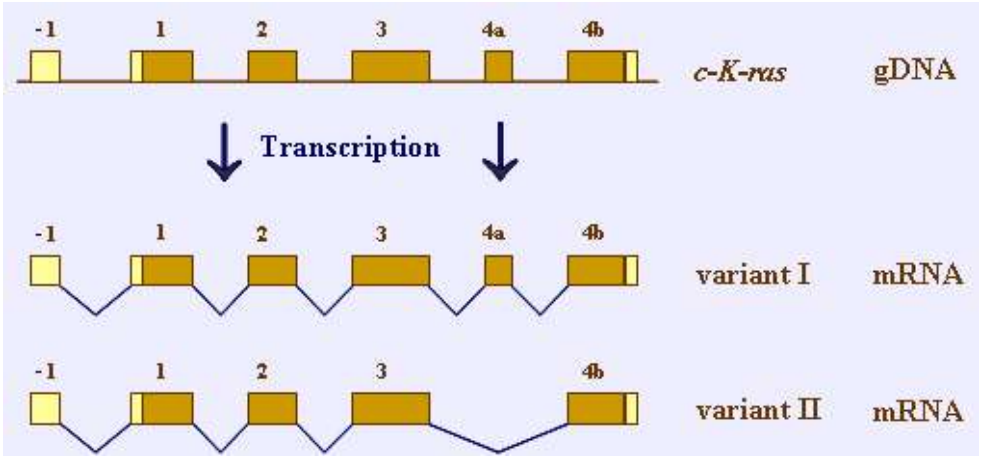

Figure 4: KRAS gene homolog [55]

1.2.2 Kras and cancer

The first oncogenes identified in human tumors were *KRAS*, *NRAS*, and *HRAS*. Mutations of KRAS have been identified in a mutable rate [56]. RAS activating mutations are found in acute leukemia with frequency of 12- 44%. [57]. These mutations are not associated

to a specific karyotypic abnormalities, clinical features or prognosis leukemia (X. Thomas & Elhamri 2005a) and occur mostly in exon 12, 13 and 60 **(**Figure 5) [58].

Mutations in *RAS* genes may be induced by carcinogenic agents, such as nicotine gas. This implies that the type of mutations found may provide information about the type of mutagen involved in the induction of the mutations and, consequently, in the induction of the tumor.

Two codons in the *KRAS* gene are mainly known to generate alternated proteins that are constitutively activated without the signal of a ligand bound to the EGFR. This are the codons 12 and 13 in the exon 2 of the *KRAS* gene. Both codons encode the amino acid glycine in the wild type protein. Replacement of one of the first two bases leads in both codons to an amino acid exchange in the KRAS protein, resulting in resistance of the tumor to the treatment [58].

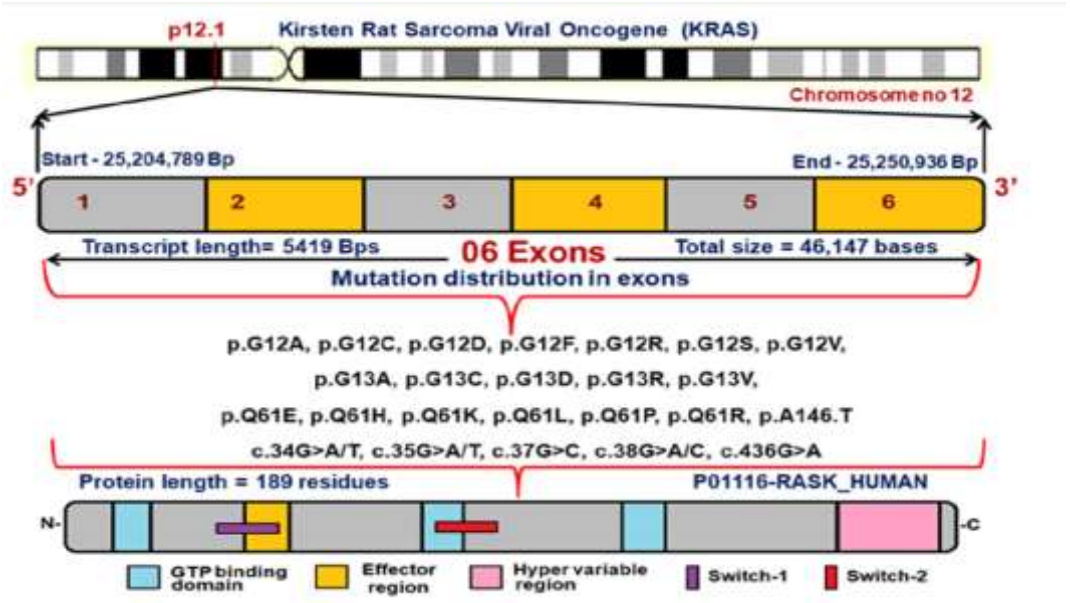

Figure 5: Exon 12 structure of KRAS gene with reported mutations and their protein domain [55]

2 Gene alteration in ALL

ALL is based on a number of recurring chromosomal abnormalities, including hyperdiploidy, hypodiploidy, translocations t(12;21)(ETV6-RUNX1), t(9;22)(BCR-ABL1),

and t(1;19)(TCF3-PBX1), and rearrangement of the mixed-lineage leukemia (MLL) gene [59].

ALL B: is characterized both by a blockage of B-cell differentiation and uncontrolled proliferation of blastic cells. Adult and childhood B-ALL differs in terms of prognosis. Cure rates now approach 90% and 40 % for pediatric and adult ALL, respectively. Both pediatric and adult patients face significantly inferior outcomes if the disease recurs [60].

ALL T: the most frequent ones are: t(10;11)(q25;p15)NUP98/ADD3;t(10 ;14(q24 ;q11)/NHL (T-ALL ;NKFB2 and TCRA-D, not rare) ; t(10 ;10)(p12 ;q21)CTNNA3/ARHGAP21 [61].

Pratical Part

Patients and method

1 Patients

Newly diagnosed AML patient who were admitted to the University hospital Center of Constantine (CHU), hematology department were our target study population. The samples were obtained from a DNA repository from a prior research on the genetics of Acute Leukaemia. In fact, we were unable to study new cases due to The COVID-19 pandemic.

A total of 31 individuals with acute leukemia were included in our research. Sex, age, complete blood count, bone marrow Biopsy and conventional karyotype, if available, were the clinical and biological criteria gathered.

The following criteria were used to enroll patients for this study:

1.1 **Exclusion Criteria**

- Hematological malignancies other than acute leukemia.
- Pediatric malignancies were excluded.

1.2 **Inclusion criteria**

- Acute myeloblastic and lymphoblastic leukemia.
- All patients were newly diagnosed and never received chemotherapy before.

All patients were informed about the study and provided written consent.

2 Methodology

2.1 **Biological analysis**

After studying the available patient records, sorting data and collecting information, we have focused on the main biological criteria that are needed for the diagnosis which are:

Haemogram (complete blood count): is a series of tests made on the blood circulating cells, it can assess number of illnesses and disorders, including infections, anaemia, and leukaemia.

The myelogram: is a haematological examination both quantitative and qualitative cytology of the spinal cord after aspiration of the bone marrow. It is important to confirm the diagnosis and identify the type of leukaemia.

Immunophenotyping: flow cytometric immunophenotyping (FCI) is a critical component that enables thorough evaluation of important surface-membrane and

intracellular antigens expressed by leukemic cells, thus facilitating blast identification and phenotypic characterization.

2.2 **Molecular analysis**

The molecular study included two main steps:

- DNA isolation
- Mutational analysis

All procedures were done within the National Research Center of Biotechnology (crbt).

Equipment

- Micropipettes and tips,
- Eppendorf®,
- Skirted well plates
- Vortex,
- Centrifuge,
- PCR tubes,
- - Thermocycler (BIO-RAD® iCycler)
- Gloves,
- Support for molding,
- Erlenmeyer
- Microwave,
- Electrophoresis tank and comb,
- Plates for coloring,
- Pipette,
- Power generator,
- Room with UV lamp equipped with a photograph-printing system.
- Casting tray.

2.2.1 DNA isolation

Like it was mentioned before, our samples were obtained from a prior study, so the DNA was already extracted according to the “Slting-out” method [62]. Meanwhile, during our internship in the crbt, we have been able to attend the DNA isolation technique. We will describe below the main steps.

After collecting Blood samples in 5 ml EDTA tubes. There is 3 basic steps for DNA extraction; the preparation of leukocytes, the extraction of the DNA itself and finally the solubilization.

- Preparation of leukocytes: Leukocytes are separated from whole blood by hypotonic lysis of erythrocytes in Tris-EDTA buffer (20 mm Tris, 5 mm EDTA, ph 7.5) (TE) 20: 5 for 10 minutes in ice. After washing, the pellet is resuspended in TE 20: 5.
- DNA extraction: is done by adding a lysis buffer (400 mm nacl, 2 mm EDTA, 10 mm Tris, ph 8.2), 10% Sodium Dodecyl Sulfate (SDS) and proteinase K at 10 mg / ml. The tubes are rotated on a wheel, at 27 ° C, overnight, and are cooled the next day in ice for 5 minutes. Then 1 ml of 4M nacl is added to allow the release of nuclear DNA in the lysate as well as the digestion and elimination of the proteins associated with it by precipitation. The DNA ball is formed in the supernatant by precipitation with pure ethanol. Once the DNA ball has been collected with a Pasteur pipette, it is rinsed twice in 70% ethanol and then placed in a 1.5 ml Nunc® tube.
- Solubilization: the DNA thus obtained is dissolved in aqueous phase by adding between 300 and 1000 µl of bi-distilled water depending on the size of the ball. It is left overnight on a rotator at 37 ° C, then at room temperature until complete dissolution. This operation lasts between 1 and 2 days.

2.2.2 Mutational analysis

A. Amplification of DNA by PCR

- **Principle**

The polymerase chain reaction (PCR) is a molecular biology method that amplifies a specific segment of DNA in many orders and produces thousands to millions of copies needed for various purposes.

The PCR technique is bases on the natural processes a cell uses to replicate a new DNA strand by using a DNA-polymerase enzyme, template DNA, primers, nucleotides (A, T, C, and G). As a primer is attached to one of the DNA strands in the particular location selected to begin the synthesis, the enzyme synthesizes a complementary sequence of DNA

using The nucleotides. Primers restrict the sequence that may be duplicated, resulting in the amplification of a particular DNA sequence with billions of copies.

PCR is a three-steps process that is carried out in multiple cycles, the initial step is denaturation followed by hybridization and finally the extension.

- **Detection of the FLT3-ITD mutation**

The *FLT3*-ITD consists of in-frame insertions of duplicated sequences, most of them are located in the juxtamembrane domain, these duplications are easily identified by PCR.

1 Preparation of FLT3-ITD PCR mixture

We amplified by PCR exons 14 and 15 of the FLT3 gene (OMIM : 136351) using a pair of specific primers (Table 01) .

Table 01: The primers (F) and (R) used of exons 14-15 of the FLT3 gene

Primers	Sequence (5 '→ 3')	Amplicon size (bp)
14 (F)	**GCAATTTAGGTATGAAAGCCAGC**	**389**
15 (R)	**CTTTCAGCATTTTGACGGCAACC**	

The PCR reaction mixture was prepared at room temperature and the components of the master mix used for the amplification are listed in (Table 02).

Table 02: Composition of the PCR reaction mixture for the amplification of exons 14 and 15 of the FLT3 gene

Reagents	Initial concentration	Concentration	Volume (µl)
H2O			16.8
Buffer (-MgCl2)	10X	1X	2.5
MgCl2	2.5mM	2.5mM	2
dNTPs	25mM	10mM	0.5
Oligo F	100mM	10mM	0.5
Oligo R	100mM	10mM	0.5
Taq polymeras (biomatik)	5U/µl	1U	0.2
ADN		50ng	2
	Total		25

2 Progression of the PCR cycles

The cycles corresponding to the different temperatures of the PCR were programmed in a thermal cycler (BIO-RAD® icycler) in number of 35 cycles; the amplification process is presented in (table 03)

Table 03 : Thermal cycler program for the amplification of exons 14-15 of the FLT3 gene

Process	**Temperature (° C)**	**Time**	**Number of cycles**
Initial denaturation	94	4 minutes	1
Denaturation	94	30 seconds	35
Hybridization	56	30 seconds	
Elongation	72	1 minute	
Final elongation	72	10 minutes	1

3 PCR Product Observation

The PCR products are submitted to an electrophoresis in an agarose gel to confirm successful amplification of the exon **14** and **15.**

The size of the amplified fragments is checked by electrophoresis on a 3% agarose gel by adding 3g of agarose to 100 ml TBE (Tris Borate EDTA) added to 10 µl of gel stain which is an intercalant agent used to emit fluorescence during the electrophoresis observation. We put in the first well 3µl of size marker (PM 100 bp LADDER). Then, in the rest of the wells, 10 µl of amplification product are added to 6µl of the SYBR Green dye which is a highly sensitive reagent for staining DNA in electrophoresis.

After migration, the gel is subjected to UV light. The amplified fragments may be observed as fluorescent strips of the same or different sizes.

The expected size of fragments in the PCR analysis of FLT3-ITD gene codon 14 & 15 are:

	Normal allele	**Mutant allele**
FLT3-ITD codon 14 & 15	389 pb	>389 pb

- **. Screening for k-ras gene mutation**

The k-ras gene (OMIM:190070) point mutation in codon 12 is detected by the PCR-RFLP technique.

1 Preparation of the k-RAS PCR

We used a pair of specific primers as mentioned in (Table 4).

Table 4 : The primers (F) and (R) used of exons 12 of the k-RAS gene

Primers	Sequence (5 ’→ 3’)	Amplicon size (bp)
k-ras (F)	ACT GAA TAT AAA CTT GTG GTA G‘IT GGA CCT	157
k-ras (R)	TCA AAG AAT GGT CCT GGA CC	

The reagent used in the PCR process as well as the amounts needed for each tube are listed in the (table 05). The PCR reaction mixture was prepared at room temperature.

Table 05: Composition of the PCR reaction mixture for the amplification of exons 12 of the k-RAS gene

Mix	Initial concentration	Final concentration	Volume (µl)
Buffer	10X	1X	2.5
MgCl2	2.5mM	2.5mM	0.75
dNTP	25mM	10mM	3.12
Primer F	100mM	10mM	2
Primer R	100mM	10mM	2
Taq polymerase (biomatik)	5U/µl	1U	0.4
H2O			13.23
ADN		50ng	1
Total :			25

2 Progression of the PCR cycles

The cycles corresponding to the different temperatures of the PCR were programmed in a thermal cycler (BIO-RAD® iCycler) in number of 35 cycles (Table 06).

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Table 06: Thermal cycler program for the amplification of exon 12 of the k-ras gene

Process	**Temperature (° C)**	**Time**	**Number of cycles**
Initial denaturation	94	4 minutes	1
Denaturation	94	30 seconds	35
Hybridization	56	30 seconds	

Elongation	72	1 minute	
Final elongation	72	10 minutes	1

3 PCR product observation

The pcr results are electrophoresed using an agarose gel to ensure that the exon 12 is amplified successfully.

To observe the pcr products, we used a similar procedure to the one used for the flt3-itd. We prepared a 3% agarose gel, deposited the amplified products, and then observed the results.

4 The enzymatic digestion

- **Principle**

The PCR products are subjected to enzymatic digestion, which involves the molecules DNA being cleaved at specific locations known as restriction sites. A mutation in the restriction site sequence can make a particular restriction site vanish or emerge. Variations in the number and length of restriction fragments produced after enzymatic digestion are used to identify these DNA changes.

- **kras sequence and restriction site of BstNI enzyme**

Mutations at the first and second bases of codon 12 of the K-*ras* involving replacement of a G with a C **(**Figure 6).

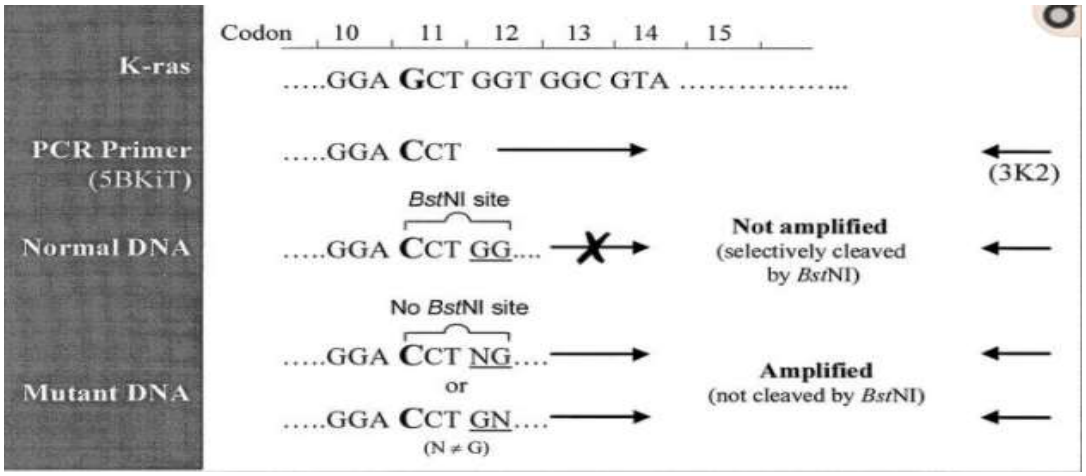

Figure 6: a schematic depiction of the k-ras sequence and restriction site of BstNI enzyme [63]

Expected sizes of fragments in PCR analysis of kras gene codon 12:

	Fragment size	Normal allele	Mutant allele
Kras codon12	157	114,29,14	143,14

- **The digestion Protocol**

Digestion was performed using: BstNI restriction enzyme with the following protocol:

Table 7: reaction mix used for the digestion with BstN1

Reagents	volume
PCR reaction mixture	10 µL
nuclease-free water	17 µL 10X
Buffer	2 µL
BstNI	1 µL

After preparation of the digestive reaction mixture (Table 7) PCR products are incubated for 3 hours at a temperature of 60°C. The digestion product have been electrophoresed using 3% agarose.

2.2.3 The statistical analysis

All our data were analyzed using Fisher's exact test and student t test. P value was considered significant if it was <0.05.

The results of all patients assigned to this study were processed using the SPSS statistical program (version 20.0).

Results and Discussion

We conducted this study for several purposes, notably: describing the biological and clinical characteristics of patients with acute lymphoid and myeloid leukemia, as well as investigating the molecular variations of two genes known to be among the most common mutations in acute leukemia.

1. Description of the study population

The patients were chosen according to their type of acute leukemia, myeloid or lymphoid, each patient underwent an individual study about the clinical-biological parameters. A total of 31 patients took part in this study: 68% of them have AML (21 patients) and 32% have ALL (10 patients) (Figure 7).

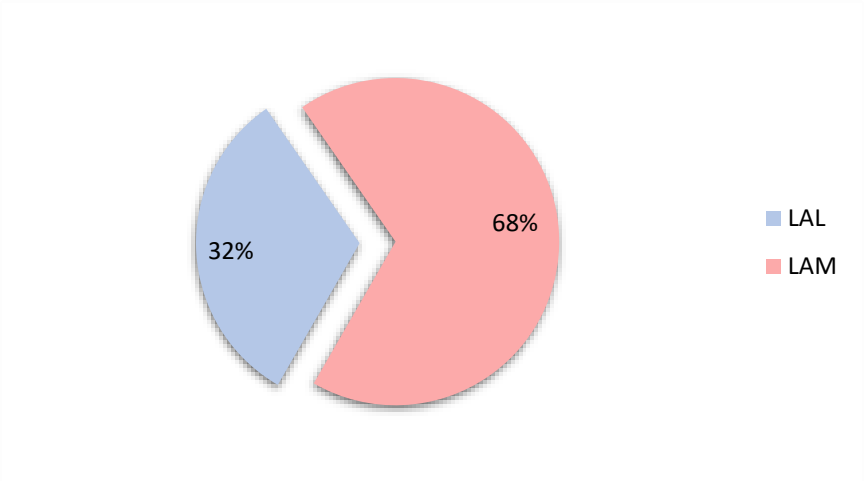

Figure 7 : frequencies of both AML and ALL cases

According to the recent data, leukemia ranked the 14th position on an international scale, while in Algeria; their incidence places them in the 11th position with a frequency of 2.9% [64].

Regarding our results, the frequency of AML is twice that of ALL (68% *vs*. 32%). The incidence of acute Lymphoid and myeloid leukemia in our study is consistent with what has been reported in the literature [65] , the most likely reason is the high prevalence of AML in adults as opposed to ALL which is more common among children under age 20 [66]. Added to that, in this study, one of the exclusion criteria was pediatric leukemia. That explains the disparity between the frequencies of AML and ALL cases [67] [68].

1.1. Distribution by sex

Male patients represented 58% (n = 18) compared to 42% (n = 13) of the female sex. To be more specific, in ALL patients, males were represented by a frequency of 70% (n = 7) and females by a frequency of 30% (n = 3) (Figure 8), the sex ratio (M / F) is 2.3. On the other hand, no big differences were registered between male and female frequencies in the AML cases, which were 52% (n=11) and 48% (n=10) respectively with a sex ratio 1.1.

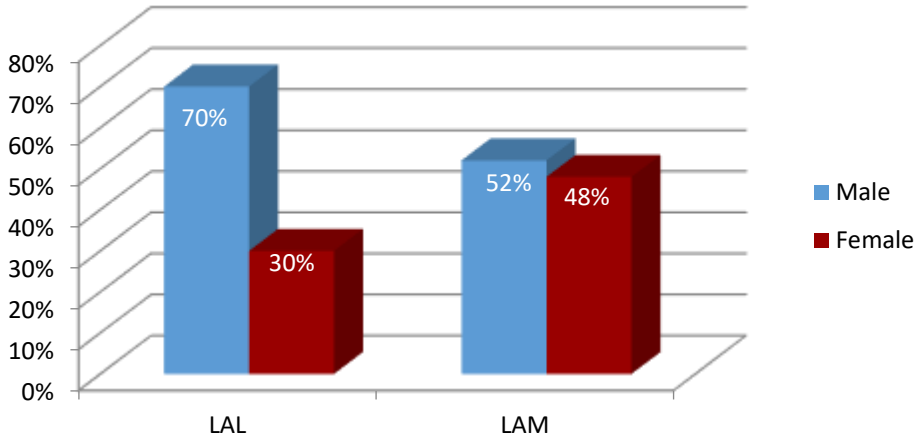

Figure 8: Distribution of patients by sex and type of leukemia

According to GLOBOCAN 2020, our results are in agreement with what was reported previously, in the USA as well as in Europe and more specifically in France; their frequencies are higher in males than in females in both LAL and LAM. [69]. These results may be explained by the fact that men are more exposed to the different environmental factors such as chemicals and exposure to ionizing radiation which may increase the risk for leukemia, another reason may be the Myelodysplastic syndrome disease, which is considered as a primary indicator of acute leukemia, and affects men more than women, in addition to some other factors like hormonal factors or genetic differences between males and females [70].

1.2. Age distribution

The mean age of the patients was 40.80±15,45years old, with extremes ranging from 17 to 86 years old, with instances prevailing between 50 and 59 years.

There was a substantial difference in age in the samples between the two types of leukemia. In particular, majority of patients under the age of 50 were ALL (90%) Whereas, most patients over the age of 50 years were AML (56.36 %).

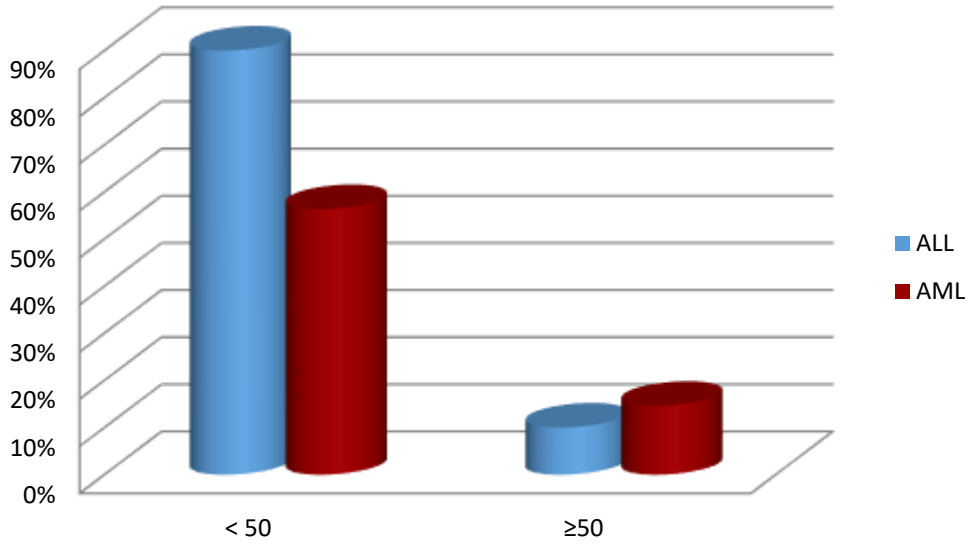

Figure 9: Age distribution

Concerning ALL patients, the mean age was 29.4±14, 65 years. Among them, 40% were under the age of 20 and only one case was over 50 years old. Contrary to the AML patients, where most of the cases had more than 50 years with a frequency that reaches 56.36%, interestingly no patient was under the age of 20 years (Figure 9).

Age is an essential factor in the incidence of leukemia. While lit affects all age groups, it differs according to the type disease. Approximately 4.8% of all leukemia and lymphoma cases have been diagnosed in people under 20 [71] [72]. ALL, which affects children and adolescents, is responsible for about 25% of pediatric malignancies. By contrast, AML is much more common in adults, with a total incidence of 3–5 occurrences per 100,000 population. Indeed, the typical age of diagnosis for AML is 66 years, with 54% diagnosed beyond 65 years of age and 33% over 75 years [73].

The incidence rate in Algeria is 17 per 100 000 individuals in those aged over 65 years. The significantly younger median age when AML is diagnosed in Algeria may reflect the relatively younger population compared to the West. In fact, we have a considerably younger age in Algeria with a median age of 45±14.2 against a typical median age of 63 years in Europe [74][75].

Depending on where the people were examined, this discrepancy may be due to the groups' regional differences or ethnic origins.

1.3. Geographical distribution

patients from mila represented the highest percentage with 32.25% (n = 10) followed by those from constantine with 29.03% (n = 9) and the lowest percentage is the one from biskra 3.22% (n = 1) (Figure 10) .

the diversity of geographical distribution is an essential factor in the study of any pathology, especially cancers, the chu of constantine is considered as a reception center for patients with acute leukemia in eastern algeria because of the lack of services in other hospitals. But that does not mean that it is the only cancer center in the eastern area.

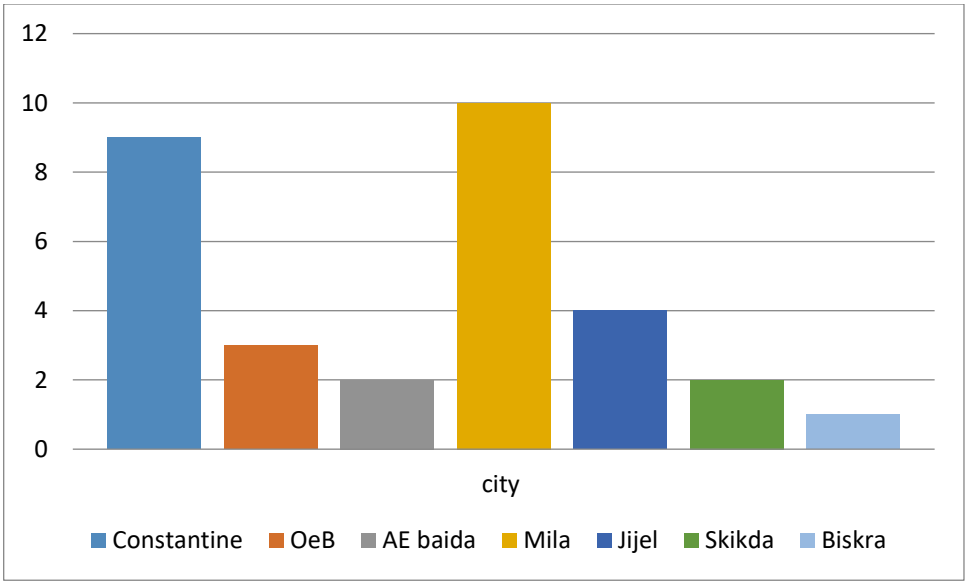

Figure 10: Geographic distribution by province

2. Biological analyzes

2.1. Hemogram

After collecting data from the patients' files, the following results have been observed.

A. White cell blood count

We noticed that only 12.9% (n= 4) of our cohort had normal WBC (the standards are: 5.2-12.4*10^9/L). The rest of them had either leukopenia (16.13%) or leukocytosis (70.97%) (Table 8).

Table 8: Leukocytosis results of all patients

Leukocytosis ($x10^9$/L)	Number of cases	Proportion %
Leukopenia : < 5.2	5	16,13 %
normal : 5.2-12	4	12,90 %
Leukocytosis : >12-99	19	61,29 %
hyperleukocytosis : ≥ 100	3	9,68 %
Total	31	100 %

B. Hemoglobin

93.38% of the patient suffer from anemia, which is the first indicator that leads to acute leukemia, confirm it with their analysis.

C. Platelets

According to the results, all patients suffer from thrombocytopenia; we noted that all the results were below the normal standards of platelets in the human body.

We conclude from the above that regardless of the type and subtype, acute leukemia patients have hyperleukocytosis, anemia and thrombocytopenia. These results may be explained by the fact that the abnormal activities that occur in the bone marrow and blasts proliferation greatly affect the components of the blood by increasing the number of abnormal, diseased and harmful cells, and in turn, the disruption or death of normal functional cells.

2.2. Immunophenotyping

Since this analysis is very expensive in Algeria, it is not affordable for all patients. In the present study, 61.29% (n=19) were able to do it. The results helped to classify the ALL into ALL B with 6 cases and ALL T with 1 case, while the rest was AML type.

There was a strong expression of markers of immaturity CD34 and HLA-DR for all cases regardless of the subtypes. Therefore, in the ALL cases, there was a large expression of

antigens like ccd79, CD19 and CD10. As for one case there is an expression of ccd3, CD5, CD7, CD2 and CD8.

Furthermore, the expression of other antigens allow to classify the cases in more specific cell lineages affected by leukemia, as we observed in the B line the presence of CD19, CD22 and CD24. Interestingly, it appears that CD20 is expressed late stage followed by CD10.

As for the T line, the cells have the same degrees of progression and development of the disease because of the expression of the antigens CD7 in an early phase, then the CD2 and CD5 antigens appear on the surface of the T precursors.

As for the cases with AML diagnosis, we have also strong expression of CD34 and HLA-DR and precisely the presence of CD13 and CD33 that are highly expressed compared to Myeloperoxydase CD117, CD33, CD13, CD11, CD14 and CD36.

The use of immunophenotyping was suggested by the World Health Organization (WHO) [76], as it was found that the study of Cluster of Differentiation (CD) is a necessary step for studying acute leukemia and identifying the different subtypes. Immunophenotyping is a necessary key in the diagnosis because it help to determine the subclasses of ALL according to the EGIL classification [77].

These observations are in line with what has been reported previously [76] [78]. According to the WHO, the surface molecules that are always expressed in the hematopoietic cells are ccd3 and CD7 In addition to recurrent expression of CD8 and CD4, as it was noted that the rest of antigens, such as CD2, CD3, CD4, CD5, CD8, and CD10 are variably expressed [78].

All the antigens mentioned above are forms of the presence of cell blasts in different stages that have not reached a maturation phase thus confirming the blastic status that has invaded the peripheral blood of ALL patients and making an accurate diagnosis for each case.

2.3. Myelogram and aml classification

The myelogram is the essential part of the diagnosis by determining which cell lineage had invaded the bone morrow.

In our cohort, most of the patients (87.09%) had blasts over 50% regardless of thy subtype such as erythroblasts, myeloblasts, lymphoblasts..., which is a sign that the blasts are more present than the mature cells in the bone morrow. These results are the first tool to confirm the diagnosis of leukemia.

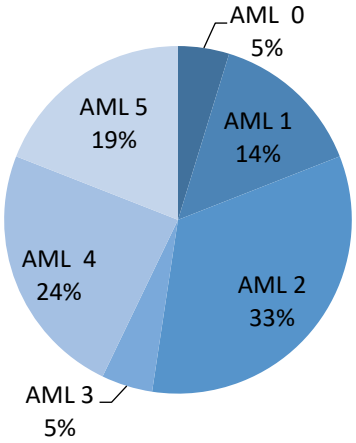

Figure 11 : FAB classification of AML patients

Within AML patients, a predominance of AML2 subtype with a frequency of 33,33% (n = 7) was registered, followed by AML4 23,81% (n = 5) and AML5 19,05% (n = 4) then AML1 14,29% (n = 3) finally LAM 3 and AML0 4,76% (1 cases for each) (Figure 11).

2.4. Karyotype

It is worth mentioning that not all the patients can afford this analysis due to its costs, 58.06% (n=18) of them had the cytogenetic results. Among them, 50% (n = 9) had a normal karyotype.

The cytogenetic plus the myelogram and the immunophenotyping certainly gives the best results. Since cytogenetic analysis is the most important diagnostic tool for determining prognosis in acute myeloid leukemia [79].

A. For the AML

Among the results obtained, acquired clonal chromosomal abnormalities were found in 40% of patients with de novo AML in our cohort, the rest of them had normal karyotype.

Cytogenetic finding in AML could be divided into three groups: favorable risk, intermediate risk, and unfavorable risk. There were 30 % cases in the favorable risk group and cytogenetic abnormality includes cases with the t (8; 21), inv (16), and the t (15; 17). There

were 60% (n=6) cases in the standard risk group which includes cases with normal karyotype. The unfavorable group includes 10% (n=1) with a complex karyotype.

Consistent with the findings of other international reports, in our study, 50% of the patients showed karyotype abnormalities. Clonal chromosomal aberrations are not detected in 50% of AML patients [80].

About 60% of AML patients in our study had a normal karyotype by cytogenetic analysis. Studies from other countries have reported a normal karyotype in AML with a frequency of about 35%–55% [81][82].

The cytogenetically normal karyotype in AML is considered an intermediate cytogenetic risk group because of varying response to treatment, achievement of CR, and relapse rate. These patients should be investigated for molecular genetics alterations.

B. For the ALL

In the present study, abnormal karyotype were found in 57.15% of the cases with t (9; 22), t (8; 21), t (4; 11) and a complex karyotype. *Usvasalo et al*, detected chromosomal aberration in 85% of the cases [82]. Silva et al. Detected abnormal Cytogenetic in 92.3% of their patients [83].

These differences between our results and the literature may be due to ethnicity and geographic factors or even the limited number of our ALL cases.

Just like the AML, ALL abnormalities help divide patients in groups, for instance the presence of t (4; 11) is a bad sign for patients, it is classified in the adverse group. Indeed this translocation is considered a poor high-risk factor.

To sum up, these aberrations can be correlated with cytomorphology and immunophenotyping results, which make them a very powerful tool for both the diagnosis and prognosis of acute leukemia patients.

3. Molecular results

3.1. FLT3

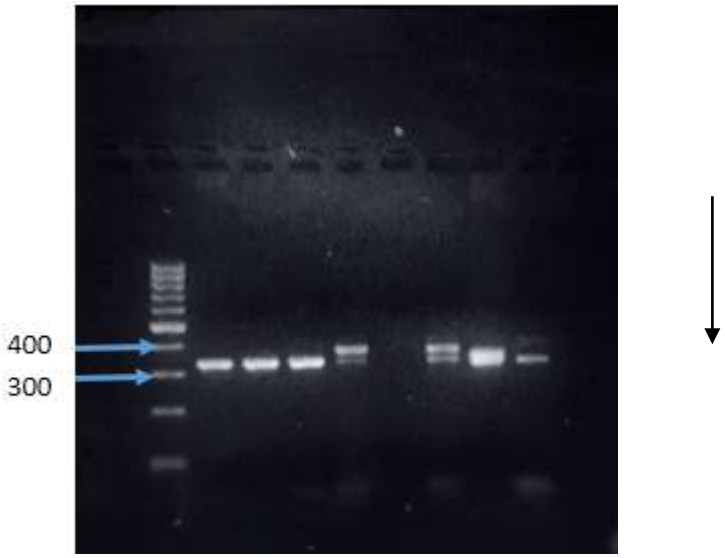

Figure 12: The genotyping of FLT3-ITD mutation

The genotyping of FLT3-ITD mutation had shown that the duplication is present in 4 out of 31 patients with a frequency of 12.91%, all the mutated patients were heterozygous (Figure 12).

In the present study, we determined the incidence of FLT3 12.91% in the adult cohort, which is lower than what is reported in the literature 20-35%. [84][85].

Contrary to our findings, the Mexican population had reported a higher frequency of patients with the FLT3-ITD mutation (20.3%) [85].

In contrast to the previous investigations, the American population has a lower frequency of patients with the FLT3-ITD mutation (1.12%). Otherwise, there were remarkable differences about the median age between American and our population [86].

In the FLT3-ITD positive group, we have observed that all the cases were de novo AML patients, the mutation was not found in any ALL case. 50% (n=2) of them were males. Concerning the non-mutated group, the males represented 59.26% (n=16) and the females represents 40.74% (n=11), the statistical analysis didn't show any significant correlation between the sex and the presence of the mutation (p=0.515). The FAB types of AML among the mutated group were M1, M2, M4 and M5.

The mean age of the mutated group was 49, while the non-mutated group had a mean age of 39.4, the *p* value was 0.266. Those results are in agreement to what has been reported in Morocco [87] and in South Africa [88], but are in contrast with an American study where the median age of the FLT3-ITDmut was over 60 [89].

3.2. K-Ras

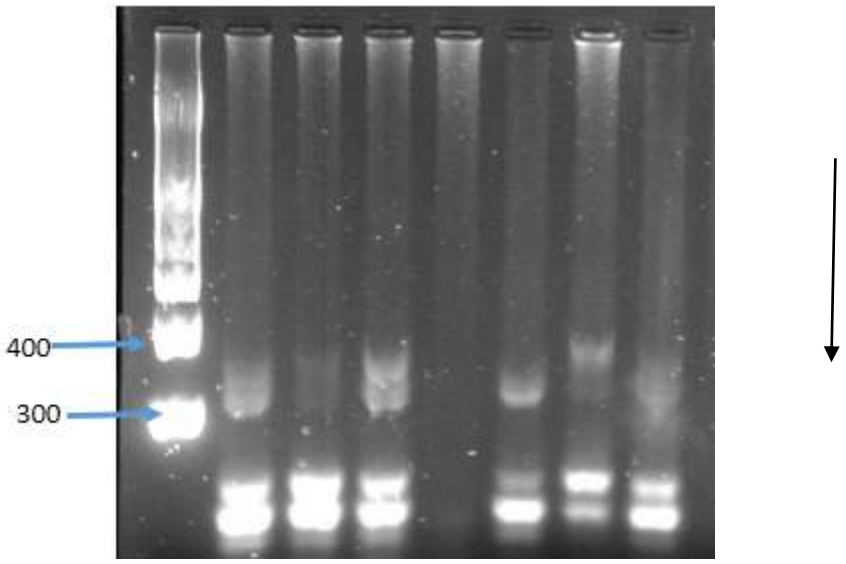

Figure 13: Mutations in K-RAS

Mutations in K-RAS were detected in 7 leukemic patients with a frequency of 22.58 % (Figure 13).

In the mutated group, 71.43% (n=5) were males and 28.57% (n=2) were females; regarding the patients with a normal genotype, the frequency of males was 58.33% (n=14) and 41.66% (n=10) represented the frequency of females. This difference is statistically significant (p=0.0015).

In the current study, we identified the k-Ras mutation is 22.58 %, this frequency is comparable to that reported in the literature, where this alteration was detected with varying frequencies (12%–44%) and represents one of the most common genetic alterations detected in AML [90] [57].

Unexpected findings emerged from our study. A significant association was gleaned between the presence of a K-RAS mutation and the sex of patients, where it is more present in the males than the females; which is in contrast to a study where the mutation is significantly

present in more females than males [91]. Currently, we have no biologic explanation for this result.

Most of the K-Ras mutations were found among the AML patients with a frequency of 85.71%, only one patient was ALL. AML1 subtype has the highest frequency 42.86% followed by AML5 25.56%, the other subtype s were 14.29%.

The mean age of patients with k-ras mutation was 42.42, concerning the non-mutated patients, their mean age was 40.13 with a p value equal to 0.702. Our results are in agreement with the one reported in Thailand report [86]. Whereas, they are lower than the Korean and Japanese results who have shown a higher median age (55 – 51 years) [92] [93].

Conclusion and perspectives

Conclusion and perspectives

Leukemia is a malignant hemopathy caused by an uncontrolled proliferation of the hematopoietic stem cell, it is diagnosed by haematological, cytological and biological examinations. Those latter can confirm the disease by detecting the cells responsible for abnormally molecular or chromosomic.

In our study, we had no opportunity to take new cases because of covid 19. Still, it wasn't an obstacle because we used samples from a DNA pool from a prior research on the genetics of Acute Leukaemia.. We noticed that the AML incidence is greater than that of ALL with sex and age parity differences. We also made a study of the clinical and biological parameters of ALL and AML patients.

In the molecular study, we noted that k-ras were present in both AML and ALL contrary to FLT3, which was only registered in AML cases.

Activating mutations in the FLT3 gene are poor pronostic variables that adversely affect patient response and survival. The simplicity of the method for detecting these mutations and their prognostic significance make them a valuable tool for AML diagnosis and therapy improvement

Contrary k-ras acted as an independent prognostic predictor in cytogenetically normal acute leukemia patients. The prognostic value of KRAS expression has been determined in various cancers. For instance, emphasized a more crucial role of KRAS in the process of leukemogenesis, and could act as a potential therapeutic target for designing cancer gene therapy.

Diagnosis requires the use of both classical and molecular cytogenetic techniques. Their complimentary relationship enables the doctor to validate diagnoses, prognoses, therapy selection, and monitoring, thus enhancing hospital patient care.

To conclude, it is important to mention the following points:

- It is recommended that cytogenetic should be performed routinely in all acute leukemia cases. A correlation must be done with various biochemical and hematological parameters, immunophenotyping, and BM morphology.

- It is necessary, to establish and apply molecular studies and integrated them with cytogenetic studies for risk stratification at diagnosis to improve therapeutic strategies.
- Our results are preliminary and cannot be confirmatory. The size of our population is limited, so it is essential to enlarge the sample size of patients. We can use the data from another region and hematological centers. Thus allowing us to draw conclusions concerning the Algerian population characteristics.
- It is also recommended to create a national cancer registry, in order to conduct useful epidemiological studies and establish a national consensus on therapeutic management to implement new treatment strategies, such as gene Tyrosine Kinase inhibitors and other targeted molecular therapies.

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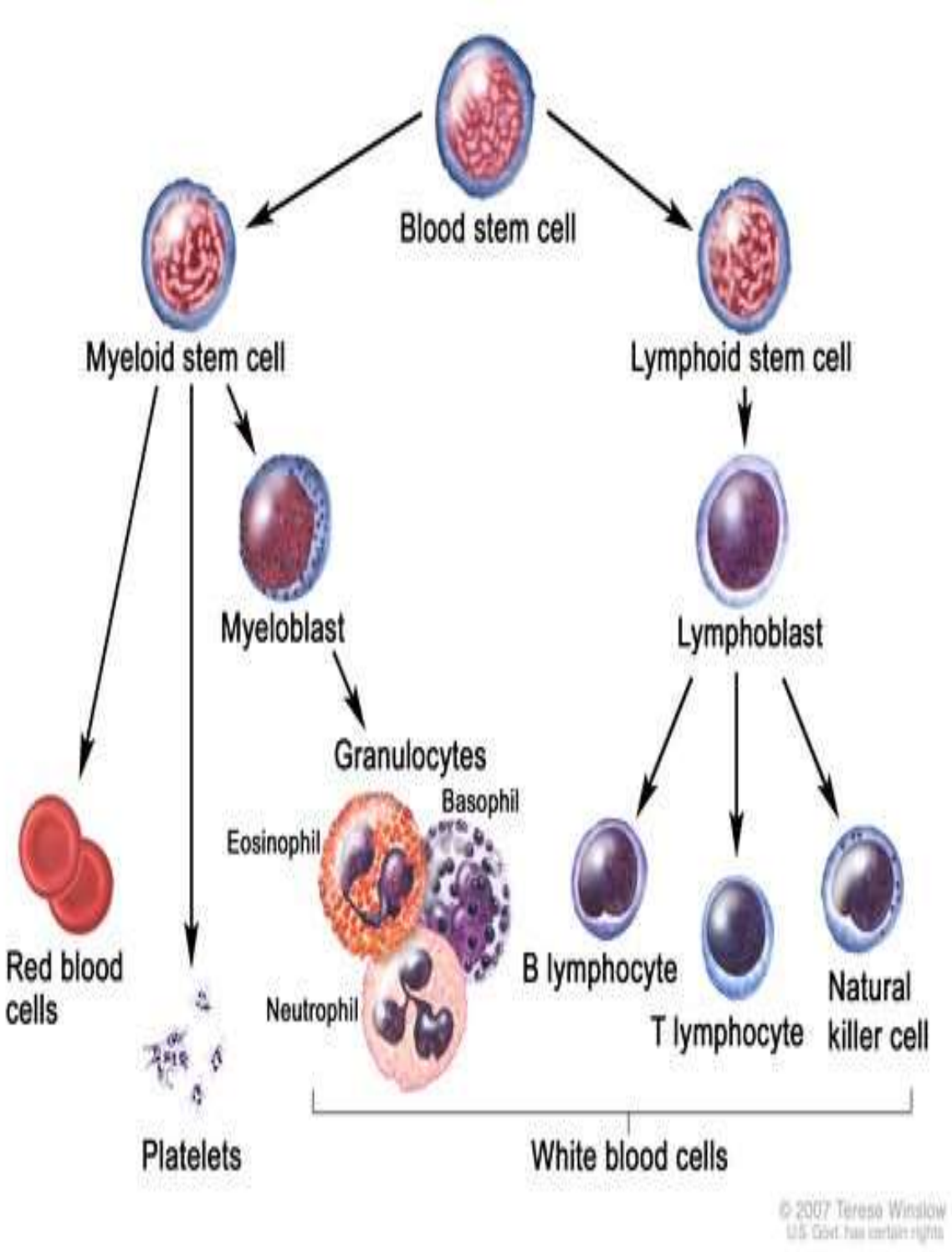
Appendix

Appendix 1 : Schematic illustration of the different blood cells types [94]



Appendix 2 : classification of the LAM according to the FAB [95]

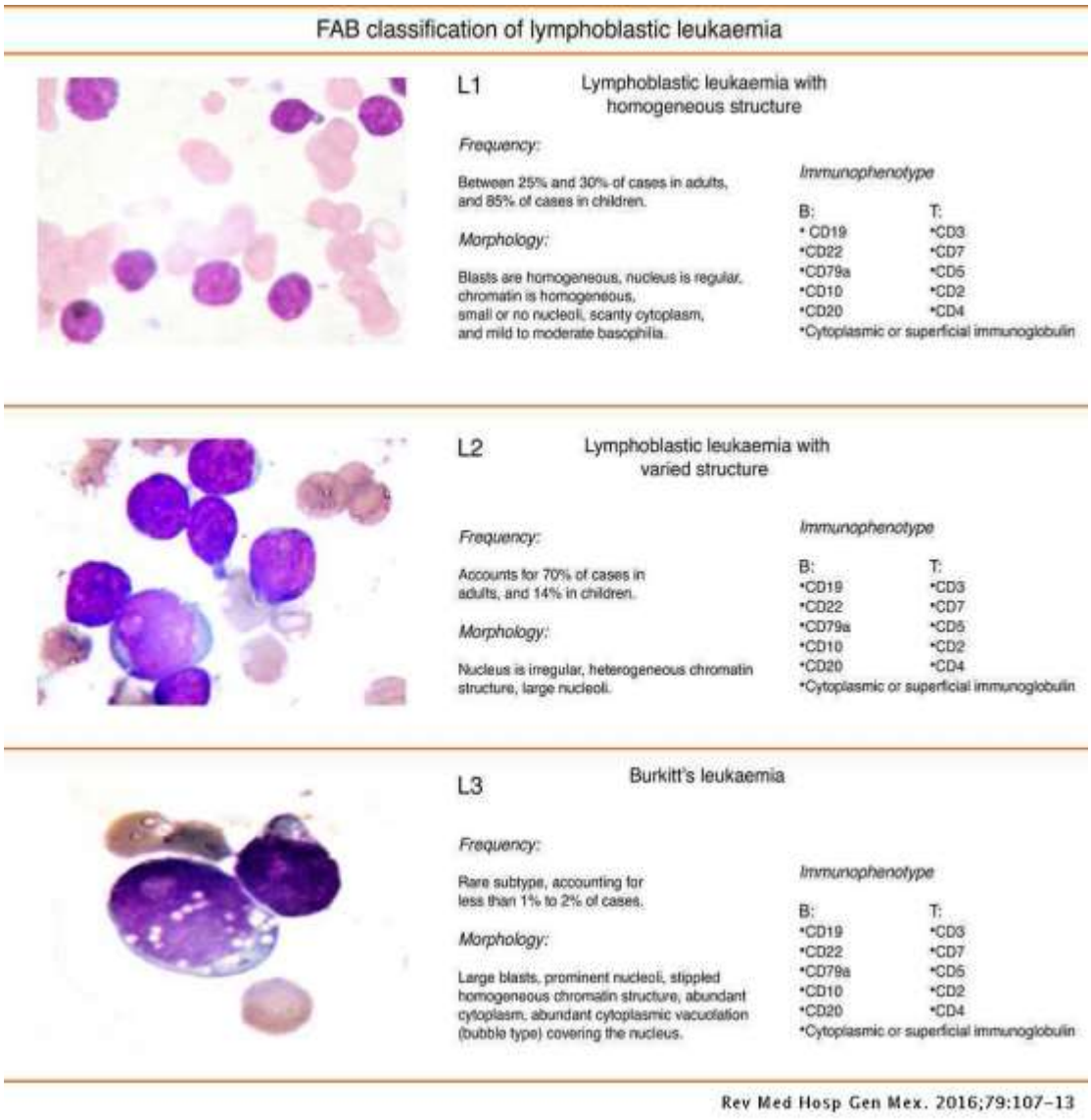
Table 1-1: The different subtypes of acute myeloid leukaemia according to the FAB classification

Subtype	Description	Cytogenetics
M0	Minimally differentiated acute myeloid leukaemia (AML).	
M1	Acute myeloid leukaemia without maturation.	
M2	Acute myeloid leukaemia with maturation.	t(8;21)(q22;q22), t(6;9)
M3	Promyelocytic, or acute promyelocytic leukaemia (APL)	t(15;17)
M4	Acute myelomonocytic leukaemia.	inv(16)(p13q22), del(16q)
M4 Eo	Myelomonocytic together with bone marrow eosinophilia	inv(16), t(16;16)
M5	Acute monoblastic leukaemia (M5a) or acute Monocytic leukaemia (M5b)	del (11q), t(9;11), t(11;19)
M6	Acute erythroid leukemias, including erythroleukemia (M6a) and very rare pure erythroid leukaemia (M6b)	
M7	Acute megakaryoblastic leukaemia	t(1;22)

Appendix 3 : the different subtypes of acute myeloid leukemia according to the WHO classification [96]

AML with recurrent genetic abnormalities	AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1* AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB MYH11* Acute promyelocytic leukaemia with t(15;17)(q22;q12); *PML-RARA* AML with t(9 ;11)(p22;q23); *MLLT3-MLL* AML with t(6;9)(p23;q34); *DEK-NUP214* AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* AML (megakaryoblastic) with t(1;21)(p13;q13);(*RBM15-MKL1*)
AML with multilineage dysplasia	Following MDS or MDS/MPD Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineage
AML and MDS, therapy-related	Alkylating agent/radiation- related type Topoisomerase II inhibitor- related type Others
AML not otherwise specified (NOS)	AML with minimal differentiation AML without maturation AML with maturation Acute myelomonocytic leukaemia Acute monoblastic and monocytic leukaemia Acute erythroid leukaemia Acute megakaryoblastic leukaemia Acute basophilic leukaemia Acute panmyelosis with myelofibrosis

Appendix 4: Subtypes of acute lymphoid leukemia according to the FAB classification [97]



Appendix 5: cytogenetic and molecular prognosis factors in AML [98]

Cytogenetic and molecular prognostic factors in acute myelogenous leukemia (AML).

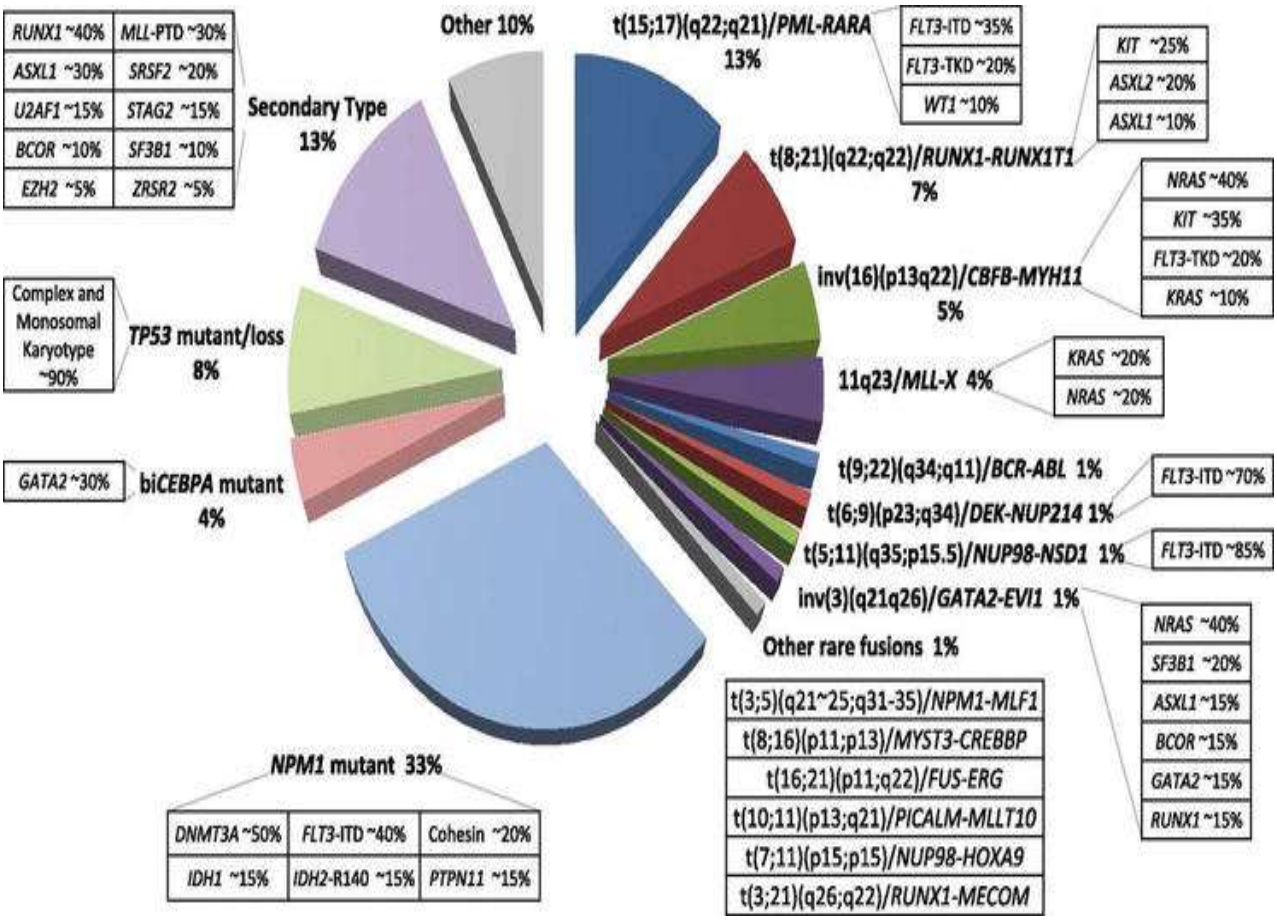
Risk category	Cytogenetics	Molecular abnormalities
Favorable risk	t(8;21)(q22;q22.1)	RUNX1-RUNX1T1
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	CBFB-MYH11
		Mutated NRM1 w/o FLT3-ITD or with FLT3-ITD^{low}
		Biallelic mutated CEBPA
Intermediate risk	T(9;11)(p21.3;q23.3)	Mutated NPM1 and FLT3ITD^{high}
	Cytogenetic abnormalities not in other category	Wild-tpye NPM1 w/o FLT3-ITD or with FLT3-ITD^{low}
		MLLT3-KMT2A
Poor risk	T(6;9)(p23;q34.1)	DEK-NUP214
	T(v;11q23.3)	KMT2A rearranged
	T(9;22)(q34.1;q11.2)	BCR-ABL1
	inv(3)(q21.3q26.2) or t(3;)(q21.3;q26.2)	GATA2, MECOM(EVI1)
		Complex karyotype
	-5 or del(5q); -7; -17/abn (17p)	Wild-type NPM1 and FLT3-ITD^{high}
		Mutated RUNX1
		Mutated ASXL1
		Mutated TP53#

Appendix 6: **Equipment of PCR**

Equipment

- Micropipettes and tips,
- Eppendorf®,
- Skirted well plates
- Vortex,
- Centrifuge,
- PCR tubes,
- - Thermocycler (BIO-RAD® iCycler)
- Gloves,
- Support for molding,
- Erlenmeyer
- Microwave,
- Electrophoresis tank and comb,
- Plates for coloring,
- Pipette,
- Power generator,
- Room with UV lamp equipped with a photograph-printing system.
- Casting tray.

Appendix7: cytogenetic abnormalities and molecular mutations found in AML [99]



Abstract

Résumé:

La leucémie aiguë est un cancer qui affecte les cellules hématopoïétiques. C'est le 15ème cancer le plus souvent diagnostiqué et la 11ème cause de décès par cancer dans le monde, il est classé selon le type prédominant de cellules impliquées comme myélocytaire ou lymphocytaire.

Dans la première partie de l'étude, nous avons analysé les données fournies dans tous les dossiers de patients atteints de leucémie aiguë, suivi d'une description de la caractéristique biologique et clinique de ces cas. Nous avons observé qu'il y avait une disparité entre les sexes concernant les deux types de leucémie aiguë ; LAM et LAL. Nous avons également noté que la survenue de cette maladie était bimodale selon l'âge.

La dernière étape était une enquête sur les variations moléculaires des gènes FLT3 et K-RAS, où nous avons enregistré une fréquence de loi des mutations FLT3-ITD et elles étaient exclusives aux patients atteints de LAM. D'autre part, la fréquence de la mutation c-K-RAS était en accord avec la littérature et a été observée chez les patients atteints de LAM et de LAL.

Dans cette étude nous avons mis en évidence la présence de mutations dans les gènes connus pour être les plus mutés dans la leucémie aiguë, nous avons également essayé d'évaluer une relation entre ces variations et les caractéristiques cliniques des patients.

En résumé, bien que l'évaluation morphologique de BM et la biopsie reste importante pour le diagnostic de la LAM, il est clair que la présence ou l'absence d'anomalies cytogénétiques spécifiques et de mutations génétiques acquises restent la pierre angulaire pour prédire le pronostic (groupes à risque favorable, intermédiaire et défavorable) ainsi que pour orienter le traitement. est recommandé d'effectuer systématiquement des études cytogénétiques et moléculaires dans tous les cas de crise aiguë. Une corrélation doit être faite avec divers paramètres biochimiques et hématologiques.

Mots clés : leucémie aiguë, LAM, LAL, cytogénétique, gène, mutation.

الملخص:

سرطان الدم الحاد هو سرطان يؤثر على الخلايا المكونة للدم. يحتل المركز الخامس عشر عالميا من ناحية التشخيص والحادي عشر من ناحية السرطانات المؤدية للموت. ويصنف حسب نوع الخلايا المصابة إلى لمفاوي أو نخاعي.

في الجزء الأول من الدراسة، قمنا بتحليل البيانات المقدمة في جميع سجلات المرضى المصابين بسرطان الدم الحاد ، متبوعًا بوصف الخصائص البيولوجية والسريرية لهذه الحالات. لاحظنا وجود تفاوت بين الجنسين فيما يتعلق بنوعين من سرطان الدم الحاد. النخاعي واللمفاوي. كما لاحظنا أن الإصابة بهذا المرض كانت ثنائية النسق حسب العمر.

كانت الخطوة الأخيرة هي التحقيق في الاختلافات الجزيئية في جينات:

FLT3وK-RAS

حيث سجلنا تكرارًا لطفرات:

FLT3-ITD

و قد كانت حصرية لمرضى سرطان الدم الحاد النخاعي. من جهة أخرى فقد كانت التوتر المسجل في طفرة البروتين

Kras

متوافق مع الدراسات السابقة وقد لوحظ عند المرضى من الفئتين سواء مرضى ابيضاض الدم اللمفاوي الحاد أو ابيضاض الدم النخاعي الحاد.

أظهرنا في هذه الدراسة وجود طفرات في الجينات المعروفة بأنها الأكثر تحورًا في ابيضاض الدم الحاد، وحاولنا أيضًا تقييم العلاقة بين هذه الاختلافات والخصائص السريرية للمرضى.

باختصار، على الرغم من أن التقييم المورفولوجي يبقى دائما مهما لتشخيص ابيضاض الدم النخاعي الحاد إلا أن وجود تشوهات خلوية معينة من عدمه هو أساس التنبؤ بالإصابة و تصنيفها لثلاث مجموعات (خطيرة و متوسطة و غير خطيرة) وكذلك توجيه العلاج. يوصى بإجراء الدراسات الوراثية الخلوية والجزيئية بشكل روتيني في جميع الحالات الحادة. يجب عمل ارتباط مع مختلف المؤشرات البيوكيميائية والدموية.

الكلمات المفتاحية: سرطان الدم الحاد. سرطان الدم اللمفاوي الحاد. سرطان الدم النخاعي الحاد. مورثة. طفرة. خلوي.

Academic year: 2021-2022	**Presented by:** Attaf Sonia Benmansour Nihel Lakehal Ayat Meissoune

Title: Contribution to the genetic study of acute leukemia in Eastern Algeria

Thesis presented for the graduation of a Master of Genetic

Abstract

Acute leukemia is a cancer that affects hematopoietic cells. It is the 15th most commonly diagnosed cancer and 11th leading cause of cancer death worldwide, it is classified according to the predominant type of cell involved as myelocytic or lymphocytic.

In the first part of the study, we analyzed the data provided in all acute leukemia patients' files, followed by a description of the biological and clinical characteristic of these cases. We observed that there was a disparity in gender concerning both acute leukemia types; AML and ALL. We also registered that the occurrence of this disease was bimodal according to the age.

The final step was an investigation of molecular variations of both FLT3 and K-RAS gene, where we registered a law frequency of FLT3-ITD mutations and they were exclusive to the AML patients. On the other hand, the frequency of c-K-RAS mutation was in agreement with the literature and were observed in both AML and ALL patients.

In this study we highlighted the presence of mutations in genes known to be the most mutated in acute leukemia, we also tried to assess a relation between those variations and the clinical characteristics of the patients.

To sum up, although morphological evaluation of BM aspiration and biopsy remains important for the diagnosis of AML, it is clear that the presence or absence of specific cytogenetic abnormalities and acquired genetic mutations remain as a cornerstone in predicting prognosis (favorable, intermediate, and unfavorable risk groups) as well as guiding the treatment. It is recommended that cytogenetic and molecular studies should be performed routinely in all cases of acute. A correlation must be done with various biochemical and hematological parameters.

Keywords: Acute leukemia, AML, ALL, cytogenetic, gene, mutations

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