People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of Guelma – 8 May 1945

Faculty of Natural and Life Sciences and Earth and Universe Sciences

(SNVSTU)

Department of Biology

Master's Thesis

Domain : Natural and Life Sciences

Sector : Biological Sciences

Speciality/Option : Molecular and Cellular Biology

Presented by

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Entitled

Identification of chromosomal aberrations in the diagnosis of certain genetic disorders.

Defended on : 06/21/2022 In front of the jury composed of :

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June 2022

Acknowledgments

In the name of ALLAH, the Most Gracious, the Most Merciful

First, Glory and praise to ALLAH, the Almighty, who enlightened me on the right path, and gave me the courage and perseverance for the accomplishment of this project after five years of hard work, which represents a significant step in my academic and professional career.

Then, I would like to thank the jury members, Dr. ABDAOUI WISSEM (President of the jury), and Dr. MERABET RYM (Examiner), who took their precious time and accepted to judge this work: I would like to express to you my highest consideration, and to thank you also for your professional qualities as well as your kindness and continuous encouragement for me.

I wish to express my sincere thanks to my Supervisor Prof. BENOUARETH DJAMEL E., who directed this work, for his patience and guidance, his valuable advice, and his intangible support: Words cannot express my great gratitude for the trust and belief you have placed in me, this endeavor would not have been possible without your assistance, so thank you for setting such high expectations for me. Also, I would like to thank you for your scientific and human qualities, for your expertise and knowledge shared with us throughout the years, and for making me passionate to learn and always curious about this field of study. You are sincerely such a great Professor, that our university is and will always be fortunate for having you as a lecturer. Please know that your influence on my life and career will affect me until the end of time, and that my success will be credited to you forever.

I would like to extend my sincere thanks to the Faculté des Sciences de la Nature et de La vie et Sciences de la Terre et de l'Univers, Université 8 Mai 1945 Guelma. I thank all Lecturers and staff of the Department for their support in various ways.

Many thanks to the Ministry of Higher Education and the Scientific Research for the international cooperation made with Sapienza University of Rome, which offered me the opportunity through ERASMUS+ to study there and learn from the best professors and conduct this research:

Special thanks to my Professor and co-Supervisor GIOVANNI CENCI, for his understanding, his consideration and his rigorous orientation to perform laboratory tests related to my project in the "Cattedra di Ematologia " Laboratory of the University Transfusion Center

under the supervision of my dear Professor MARIA ZAIRA LIMONGI whom I am also grateful for accepting me in her laboratory and giving me the opportunity to work with her great team. I had the chance to learn a lot of things from her on both professional and personal levels. So, I would like to acknowledge her for her inspiration, her availability, and her moral support.

I am also thankful to my other Professors: STEFANO CACCHIONE, IRENE BOZZONI and GAIA DI TIMOTEO for their help and kindness, their explanations and their scientific and personal support.

Last but not least, I would like to recognize the immense support that I get each single day from all my family members:

I am thankful to my grandparents "Patif & Marahima" for their endless warmth and affection, to my parents "MedChawki & Sabrina" for their unconditional love and encouragement, to my warm-hearted sister "Rania" without whom I would not have succeeded, to my two little supportive and loving brothers "Zizou & Amine" for always reminding me that I am the best sister that anyone could ask for, to all my aunts and uncles for their extreme tenderness and care, especially "Amima Chahinez" who would have offered me her eyes if it were possible, to all my cousins for their belief in me and their emotional and unwavering support, especially my sisters by heart "Melissa, Narimene & Yousra". Dear all, not everyone is so privileged to have a family like you.

My big appreciation to all my friends and close ones, for their sincere friendship and enormous back, for their consistent motivation, and for always being proud of me.

I would be remiss in not mentioning my acknowledgment to all my previous teachers and every person who has supported me by any mean or any word, and wished me good luck, especially Dr. RAMOUL ABIR who helped me a lot with her precious advice, and supported me in each of my steps. It really means a lot to me.

Encouragement and belief in me are the greatest nourishment of my soul.

With the love of all of you, I have undertaken this journey, and with the love of all of you, I will do more, Allah willing.

Abstract

Cytogenetics is the analysis of blood or bone marrow cells that focuses on chromosomal rearrangements. Human body cells, exclusive of reproductive cells, have 23 pairs of chromosomes. So, any deviation from this is considered abnormal.

In our report, we testified by karyotyping bone marrow samples, taken from seven patients diagnosed with different hematological diseases, that the development of leukemia and other blood cancer involves changes in a cell's genetic material. Metaphase cells underwent G-Banding to identify specific chromosomal rearrangements and translocations. In hematological diseases, particularly in acute leukemias, abnormal and normal karyotypes may be present in the same sample. Structural and numerical chromosomal aberrations were examined in all karyotypes performed. Certain recurrent alterations act as hallmarks of a disease, which facilitates the identification of a specific disease by a cytogeneticist. Complex karyotypes characterized by the presence of three or more abnormalities, are generally associated with poor prognosis. The combination of conventional and molecular cytogenetics would be very useful in the diagnosis and, therefore, in the prescription of specific therapy for the treatment of diseases.

Keywords: Cytogenetics, Chromosomal aberrations, Hematopathology, Karyotyping, Diagnosis, Bone marrow, Targeted-therapy.

Résumé

La cytogénétique est l'analyse des cellules du sang ou de la moelle osseuse qui se concentre sur les réarrangements chromosomiques. Les cellules du corps humain, à l'exception des cellules reproductrices, ont 23 paires de chromosomes. Ainsi, tout écart par rapport à cela est considéré comme anormal.

Dans notre rapport, nous avons témoigné par caryotypage d'échantillons de moelle osseuse, provenant de sept patients diagnostiqués avec différentes maladies hématologiques, que le développement de la leucémie et d'autres cancers du sang implique des modifications du matériel génétique d'une cellule. Les cellules en métaphase ont subi le G-Banding pour identifier des réarrangements chromosomiques et des translocations spécifiques. Dans les maladies hématologiques, en particulier dans les leucémies aiguës, des caryotypes anormaux et normaux peuvent être présents dans le même échantillon. Les aberrations chromosomiques structurelles et numériques ont été examinées dans tous les caryotypes réalisés. Certaines altérations récurrentes agissent comme des caractéristiques d'une maladie, ce qui facilite au cytogénéticien d'identifier une maladie spécifique. Les caryotypes complexes, caractérisés par la présence de trois anomalies ou plus, sont généralement associés à un mauvais pronostic. La combinaison de la cytogénétique conventionnelle et moléculaire serait très utile dans le diagnostic et, donc, dans la prescription d'une thérapie spécifique pour le traitement des maladies.

Mots clés : Cytogénétique, Aberrations chromosomiques, Hématopathologie, Caryotypage, Diagnostic, Moelle osseuse, Thérapie ciblée.

ملخص

علم الوراثة الخلوية هو تحليل خلايا الدم أو نخاع العظام الذي يركز على إعادة ترتيب الكروموسومات. تحتوي الخلايا في جسم الإنسان، باستثناء الخلايا التناسلية، على 23 زوجًا من الكروموسومات. وبالتالي، فإن أي انحراف عن هذا العدد يعتبر غير طبيعي.

خلال دراستنا، شهدنا من خلال التنميط النووي لعينات نخاع العظام المأخوذة من سبعة مرضى مصابين بأمراض دموية مختلفة، أن تطور اللوكيميا وسرطانات الدم الأخرى ينطوي على تغييرات في المادة الوراثية للخلية. خضعت الخلايا في الطور الفوقي لتقنية التصبيغ بجيمسا لتحديد ترتيب الكروموسومات وانتقالاتها. في أمراض الدم، وخاصة في اللوكيميا الحادة، قد تكون الأنماط النووية الطبيعية وغير الطبيعية موجودة في نفس العينة. تم فحص الانحرافات الهيكلية والعددية للكروموسومات في جميع الأنماط النووية التي تم إجراؤها. تعمل بعض التغييرات المتكررة كسمات مميزة لمرض ما، مما يسهل على أخصائي الوراثة الخلوية تحديد مرض معين. ترتبط الأنماط النووية المعقدة، التي تتميز باحتوائها على ثلاث تشوهات أو أكثر بشكل عام بـتوقعات سير علاج سيئة. سيكون الجمع بين الوراثة الخلوية التقليدية والجزيئية مفيدًا جدًا في التشخيص، وبالتالي في وصف علاج محدد لعلاج الأمراض.

الكلمات المفتاحية: علم الوراثة الخلوية، التشوهات الصبغية، أمراض الدم، التنميط النووي، التشخيص، نخاع العظام، العلاج

الموجه.

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List of abbreviations

3C: Chromosome Conformation Capture

3D-FISH: Three-dimensional Fluorescence *in situ* Hybridization

4C: Circular Chromosome Conformation Capture

5C: Chromosome Conformation Capture Carbon Copy

AB: Antibodies

ABL1: Abelson Tyrosine-protein kinase 1

a-CGH: array-based Comparative Genomic Hybridization

Add: Addition

ALL: Acute Lymphoblastic Leukemia

AML: Acute Myeloid Leukemia

AML-MRC: AML with Myelodysplasia related changes

APL: Acute Promyelocytic Leukemia

BCR: Breakpoint Cluster Region

CA: Chromosomal aberrations

CGH: Comparative Genomic Hybridization

CLL: Chronic Lymphocytic Leukemia

CML: Chronic Myelogenous Leukemia

CMML: Chronic Myelomonocytic Leukemia

CNV: Copy Number Variations

CO_2: Carbon Dioxide

CO-FISH: Chromosome Orientation Fluorescence *in situ* Hybridization

DAPI: 4', 6-diamidino-2-phenylindole

Del: Deletion

Der: Derived chromosome

DLBCL: Diffuse Large B-Cell Lymphoma

DNA: Deoxyribonucleic acid

DSB: Double Strand Breaks

DS: Down Syndrome

EBV: Epstein-Barr virus

EDTA: Ethylenediaminetetraacetic acid

ETV6: E26 Transformation-Specific family Variant Transcription Factor 6

FAB: French-American-British

FISH: Fluorescence *in situ* Hybridization

Hi-C: High-throughput Chromosome Conformation Capture

HGP: Human Genome Project

HSC: Hematopoietic stem cells

HTLV-1: Human T-lymphotropic virus

I(17q): Isochromosome 17q

iAMP21: intrachromosomal Amplification of chromosome 21

IDH1, IDH2: Isocitrate Dehydrogenase genes 1 and 2

Idic: Isodicentric chromosome

Ig: Immunoglobulin

IGH: Immunoglobulin Heavy Locus

IL3: Interleukin 3

Ins: Insertion

Inv: Inversion

IPSS: International Prostate Symptom Score

ISCN: International System for Cytogenetic Nomenclature

MDS: Myelodysplastic Syndrome

M-FISH: Multiplex Fluorescence *in situ* Hybridization

MGUS: Monoclonal Gammopathy of Undetermined Significance

MLL: Mixed Lineage Leukemia

ML-DS: Myeloid proliferations related to Down Syndrome

MM: Multiple Myeloma

MRC: Myelodysplasia-related changes

MPAL: Mixed Phenotype Acute Leukemia

MPN: Myeloproliferative neoplasm

NGS: New Generation Sequencing

NOS: Not otherwise specified

PBX1: Pre-B cell leukemia homeobox Transcriptional Factor 1

Ph: Philadelphia Chromosome

RAEB: Refractory Anemia with Excess Blasts

RARS: Refractory Anemia with Ring Sideroblasts

RBC: Red blood cell

RCMD: Refractory Cytopenia with Multilineage Dysplasia

RNA: Ribonucleic acid

RUNX1: Runt-related transcription factor

SKY: Spectral Karyotyping

SSB: Single strand breaks

t: Translocation

TCF3: Transcription Factor 3

t-MDS: Therapy-related Myelodysplastic Syndrome

TP53: Tumor Protein P53

Tris: Trisomy

WBC: White blood cell

WES: Whole Exome Sequencing

WGS: Whole Genome Sequencing

WHO: World Health Organization

WPSS: WHO Prognostic Scoring System

Bibliographic part

General introduction

General Introduction

The diploid human genome is composed of twenty to twenty-five thousand genes; However, haploid set is estimated to be 3.2 x 10^9 base pairs. Genes consisting of Deoxyribonucleic Acid (DNA) base pairs are located on chromosomes. Every nucleus includes twenty-three pairs of chromosomes that are always received from paternal and maternal lineages. DNA molecule, together with proteins called histones form a structure called chromatin. This means that chromatin is lower order of DNA organization whereas chromosomes are higher order of DNA organization **(Watson, 2014)**.

DNA sequences or genes, composed of serially connected nucleotides are the functional heredity units. Every gene comprises of the particular set of instructions for a particular function or protein-coding, and any change in these instructions may have serious consequences.

We call changes in the structure of a gene: a mutation. A gene mutation refers to random alterations in DNA, it may occur in somatic and reproductive cells, most often, during replication and division, as well as exposure to mutagens or a viral infection. In this case, cells can make a defective protein or do not make a protein that the body needs. Whereas, changes to the structure or number of chromosomes are called chromosomal abnormalities. They can be a missing, extra, or irregular portion of chromosomal DNA.

Chromosome abnormalities may be detected or confirmed by comparing an individual's karyotype, or complete set of chromosomes, to a species-typical karyotype via genetic testing.

These chromosomal defects can be inherited from parents or be "*de novo*". Others, can be acquired. In all cases chromosome studies are required to detect anomalies, as they often provoke malignancies because of the formation of hybrid genes and fusion proteins, deregulation of genes and overexpression of proteins, or loss of tumor suppressor genes. According to the Atlas of Genetics and Cytogenetics in Oncology and Haemathology, certain consistent chromosomal abnormalities can transform normal cells into leukemic cells such as the translocation of a gene, leading to its inappropriate expression.

After culturing cells, chromosome analysis is achievable using different tools, and this approach involves the pairing of homologous chromosomes, counting them and detecting malformations.

For this reason, the term cytogenetics appeared. The development of human cytogenetics has gained momentum during the past 20 years **(Kerndrup and Kjeldsen, 2003)**. This field combinesthe study of chromosomes, their bands, all the karyotype of a specimen, in addition of cell division as well. It reveals the nature of the chromosomal defect which is an important trait thathelps distinguish normal and cancer-causing cells.

In this work, we will present some recurrent forms of chromosomal alterations and the means of detecting them (Chapter 1), then we will deepen, by making an overview of some blood cancers where these alterations are mainly identifiable (Chapter 2). Later, in the experimental part, we explained the procedures followed to make karyotypes as well as detecting anomalies, and finally, we added our investigations and future prospects.

This work was designed to search for and detect numerical and structural anomalies in the genomes of distinct individuals, diagnosed with different diseases, with the aim of diagnosing or confirming an existent disorder, and/or estimating disease progression to other illnesses.

Chapter 1

Cytogenetics and Chromosomal aberrations

I.1. History and general information

I.1.1. Molecular genetics

Molecular genetics is defined by the interplay between three classes of macromolecule: the nucleic acid molecules, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and proteins. When organisms reproduce, the genetic material or DNA is transmitted to daughter cells from one generation to the next. The collective name for the set of different DNA molecules in an organism, cell, or DNA virus, or of RNA molecules in an RNA virus is termed as the Genome. All proteins have a polypeptide core that is synthesized using genetic information within DNA molecules (or within the hereditary RNA molecules of an RNA virus) **(Strachan & Read, 2018).**

Studying the process of inheritance of the genetic information gave rise to a branch that, although it is a part of biology, it intersects frequently with many life sciences, it is Genetics that began with the discoveries of **Gregor Mendel**, a late 19^{th}-century scientist, who was after that, considered as the father of genetics. Then, this branch has expanded beyond inheritance to studying the function and behavior of genes, it is called Modern Genetics.

I.1.2. DNA; The holder of genetic information

Looking at the way in which segments of DNA that encodes for traits, also named genes pass from one cell to another, and observing the sex-linked inheritance mechanism in Drosophila and based on **Mendel**'s work, **Thomas Hunt Morgan** argued that genes are located on chromosomes in 1911. Later, **James Watson** and **Francis Crick** determined the double helix-like structure of DNA in 1953, using X-ray crystallography. Their double-helix model had two strands of DNA with the nucleotides pointing inward, each matching a complementary nucleotide on the other strand. And according to this model, the DNA molecule consists of two strands which are connected together by hydrogen bonds and helically twisted. This structure confirmed that genetic information lies within nucleotides on each strand **(fig. 1)**.

These strands of DNA tightly wrapped around proteins called histones, making a specific structure known as Chromosomes. Therefore, genes are located on chromosomes. Human cells normally enclose 23 pairs of chromosomes or 46 chromosomes in total, that 44 ones have the name of autosomes, while the remaining pair defines sex chromosomes, XY in males and XX in females **(fig. 2)**.

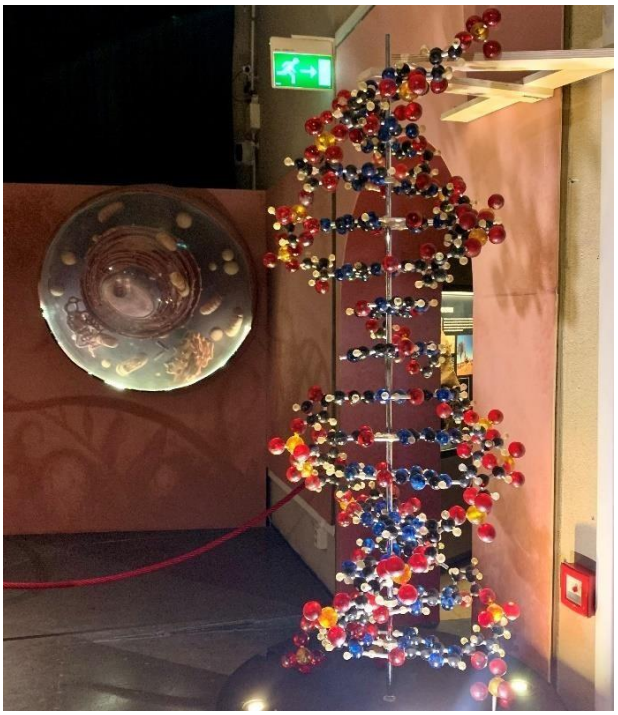

Figure 01: Watson and Crick's famous model of the double-helix of DNA

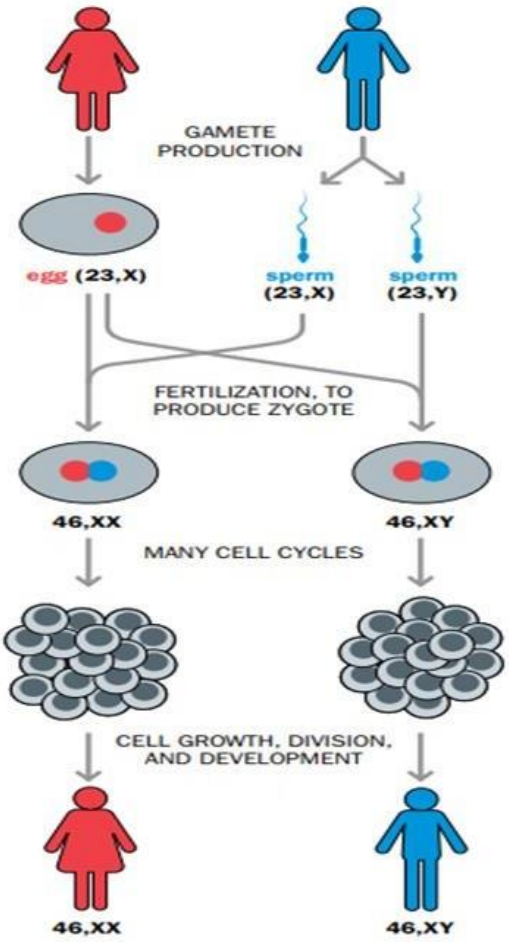

Figure 02: The human life cycle, from a chromosomal viewpoint

I.2. Introduction

The genetic information is highly conserved in sequences. During mitosis, which is the normal process of cell division throughout the human life cycle, the chromosomes segregate in a way that keeps the same information and transmit it faithfully to daughter cells to produce genetically identical products, however, meiosis reduces by half the ploidy of the cell. Both mitosis and meiosis involve chromosome replication prior to cell division **(fig. 3)**.

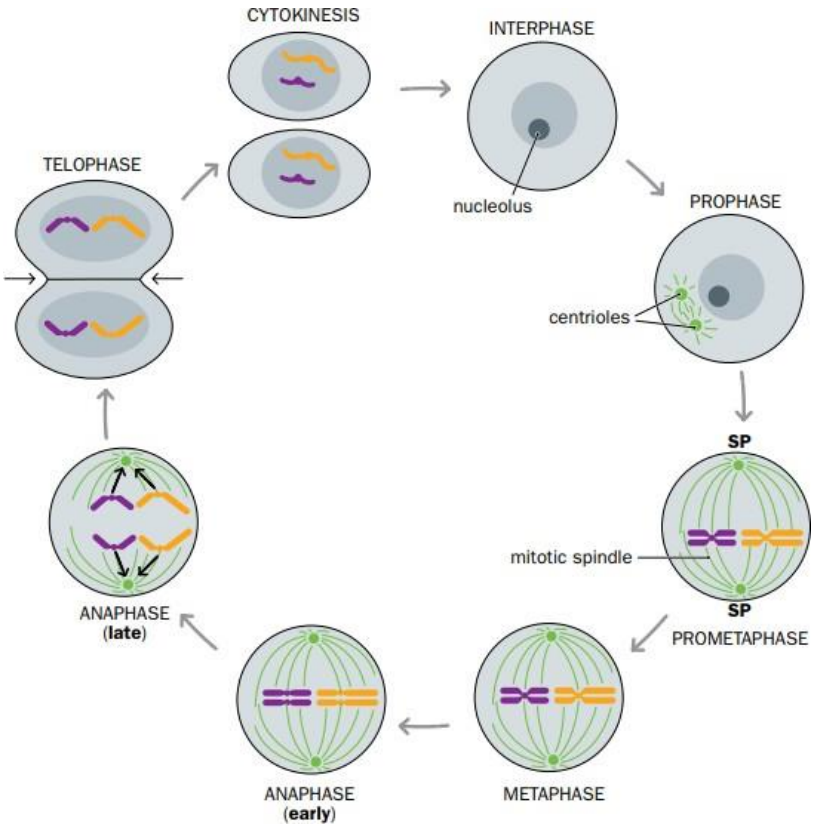

Figure 03: Mitosis (nuclear division) and cytokinesis (cell division)

Any genetic change in the number or structure will cause unwanted consequences that may lead to different diseases because they make the protein synthesis go wrong during translation. We call a change in a genetic sequence a mutation. It can include changes as small as the substitution of a single DNA building block or nucleotide base with another nucleotide base, commonly known as point mutations, or changes in crossing over that result in addition or deletion of DNA, described as chromosomal mutations. Jumping genes also cause changes in protein structure by moving their location from one chromosome to another. Mutations can be the result of errors in DNA replication during cell division, exposure to mutagens, radiation and

carcinogens, or a viral infection, in a way that can increase the risk of cancers or birth defects. Germline mutations (that occur in eggs and sperm) can be passed on to offspring, while somatic mutations (that occur in body cells) are not transmitted. Most often, mutations are repaired by specific repairing systems in specific ways, yet, sometimes, they escape the tight control, altering the structure and function of the produced protein and affecting the cell's normal activity **(Strachan and Read, 2018)**.

In this chapter, we will focus on chromosomes and see the types of changes that may happen to them and the recurrent abnormalities which can trigger divergent sorts of disorders.

I.3. Chromosomes and their identification

In the eukaryotic cell, the structure of each chromosome is highly ordered. To reach this, large negatively charged nuclear DNA molecules are bound by various proteins, including positively charged and highly conserved histone proteins and non-histone proteins. The DNA-protein complex is often called chromatin, but some non-coding RNAs may also be closely associated with chromosomal DNA **(Strachan & Read, 2018)**.

They are located within the nucleus of almost every cell in our body. At the metaphase stage of M phase, the chromosomes are so highly condensed that gene expression is shut down. This is the optimal time for viewing them under the microscope.

Each chromosome has two short arms (p arms), two longer arms (q arms), and a centromere holding it all together at the centre. Moreover, at the ends of each of our chromosomes are sections of DNA called telomeres which protect the ends of the chromosomes from degradation **(fig. 4)**.

Chromosomes can be identified by:

- Their size.
- Their shape (the position of the centromere).
- Banding patterns of their arms (produced by specific stains).

Today, researchers rely on a regularly updated mapping system known as the International System for Cytogenetic Nomenclature (ISCN) that allows them to refer to specific portions of particular chromosomes with extreme accuracy. This system is based on the use of the schematic diagram known as an idiogram, or ideogram **(fig. 5)**.

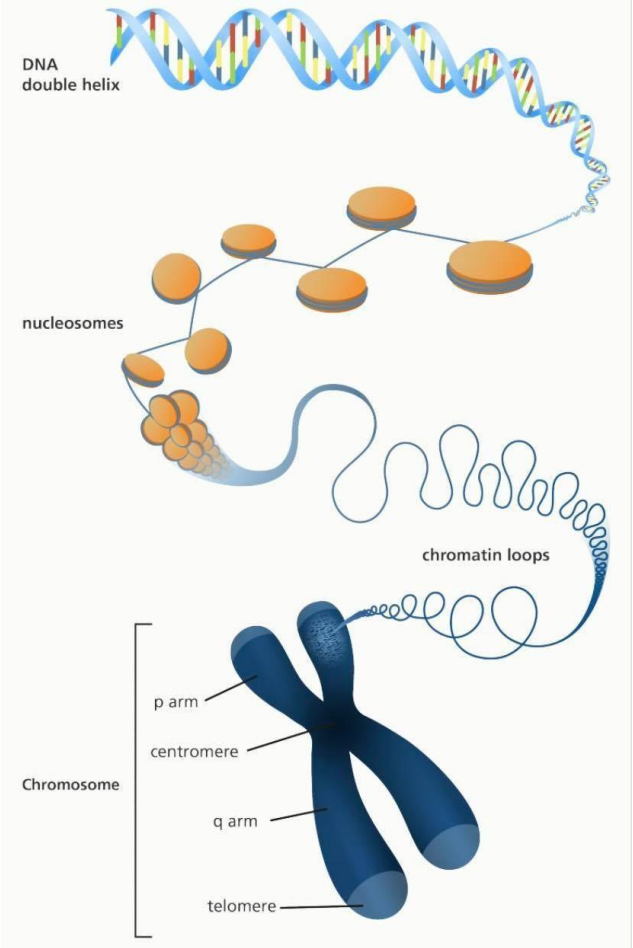

Figure 04: Illustration showing how DNA is packaged into a chromosome

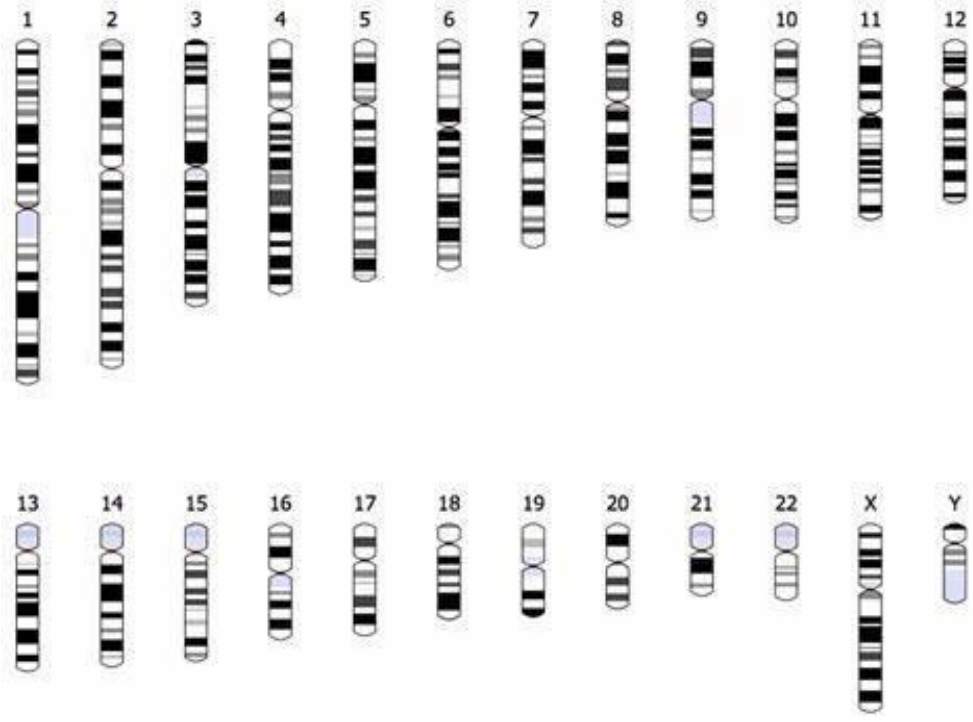

Figure 05: Idiograms of Human Chromosomes

Idiograms can be helpful in locating the positions of individual genes on chromosomes, as well as in identifying various abnormalities associated with a range of chromosomal disorders. In addition, Idiograms allow members of the scientific community to reference important sources, such as the Human Genome Project (HGP), through a universal vocabulary that can be interpreted quickly and clearly [1].

Higher resolution views of the chromosome can be obtained if staining is done while chromosomes are in prometaphase and are less condensed. As figure 6 illustrates, additional subdivisions can be detected in all of the regions of the chromosome **(fig. 6)**.

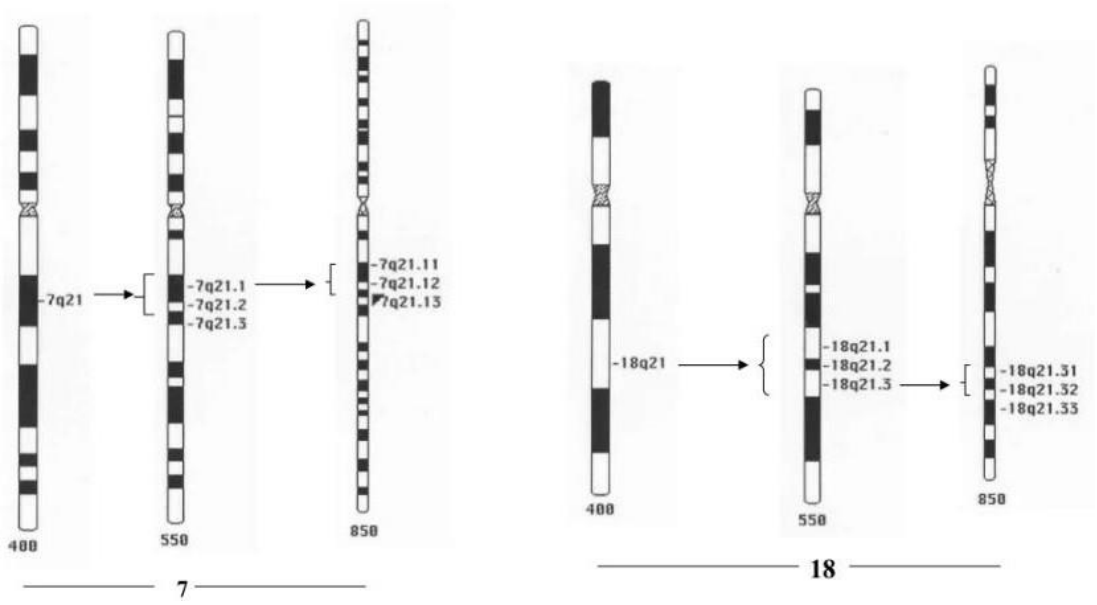

Figure 06: Low- and high-quality resolutions of Chromosome 7 and 18

I.4. Cytogenetics and karyotyping

Arnold, Flemming, and Hansemann were the first to make microscopic observations of human chromosomes in the 19th century's late years. However, it took many other years for the correct modal chromosome number to be determined in humans. Until **Eagle** developed specific culture media in 1955, the cytogenetic analysis of chromosomes depended on cells that divides spontaneously. Later, **Tjio and Levan (1956)** were the first researchers to report the correct number of human chromosomes, which is 46 using cultured embryonic cells.

The study by **Moorhead et al. (1960)** established an in vitro culture method for accumulating dividing cells using colchicine, which was first implemented in plant cytogenetics in the 1930s

(Blakeslee and Avery, 1937; Levan, 1938) to arrest cells in metaphase. In the same year, **Nowell (1960)** discovered that phytohemagglutinin has mitogenic properties; (a substance that stimulates the division of T lymphocytes in vitro), resulting in further technical improvements, like the use of peripheral blood cells.

And as chromosomal resolution has improved over the years with the development of newer techniques, human cytogenetics has evolved from a basic science into a valuable tool for diagnosing prenatal, postnatal and acquired chromosomal abnormalities.

This research area began in 1879 with the observations of the German pathologist **Arnold**, who studied carcinoma and sarcoma cells because their large nuclei made them easier to be observed. Thereby, we consider cytogenetics as a branch of genetics that focuses on the study of the structure, function, and behavior of chromosomes **(Riegel, 2014).**

Additionally, we consider two main types of cytogenetics:

1.4.1. Conventional cytogenetics

It is the study of chromosomes through G bands. It is carried out when chromosomal alterations need to be identified in terms of number and structure, such as, for example, trisomy and monosomies.

This type of study requires a certain number of metaphases and does not have the ability to detect submicroscopic changes or rearrangements of chromosomes (detects major structural abnormalities and depends upon operator skills). It is for this reason that conventional cytogenetics must go hand in hand with band analysis, Fluorescence *in situ* Hybridization (FISH) or Comparative Genomic Hybridization (CGH).

1.4.2. Clinical cytogenetics

The first science to offer a perspective of medical genetics based on human genomes, their structure and inheritance. Applied to practice, it can be very helpful in diagnosing and treating diseases. It detects diseases such as congenital malformations and haemato-oncological disorders. Through the use of certain techniques, it is possible to identify the presence of a tumor cell among 20 normal cells. The most important advantage of cytogenetic research is that all the chromosomes can be visualized at the same time **(fig. 7)**.

This type of study must be carried out when evaluating hematological neoplasms that present some kind of alteration when the diagnosis is made.

It is important along with the studies to consider the patient's history and laboratory tests. At the same time, cytogenetics can help give a characterization of the type of leukemia and its prognosis, even if we don't know the origin of the particular neoplasm. That's why it is a key in the discovery of Mendelian or monogenic hereditary diseases, multifactorial diseases, mitochondrial and chromosomal diseases. Besides, cytogenetics can also be used to identify genetic diseases that affect chromosomes while the baby is still in the mother's womb, to determine gender in genetic diseases associated with sex, and to learn whether parents are carriers of any kind of structural change.

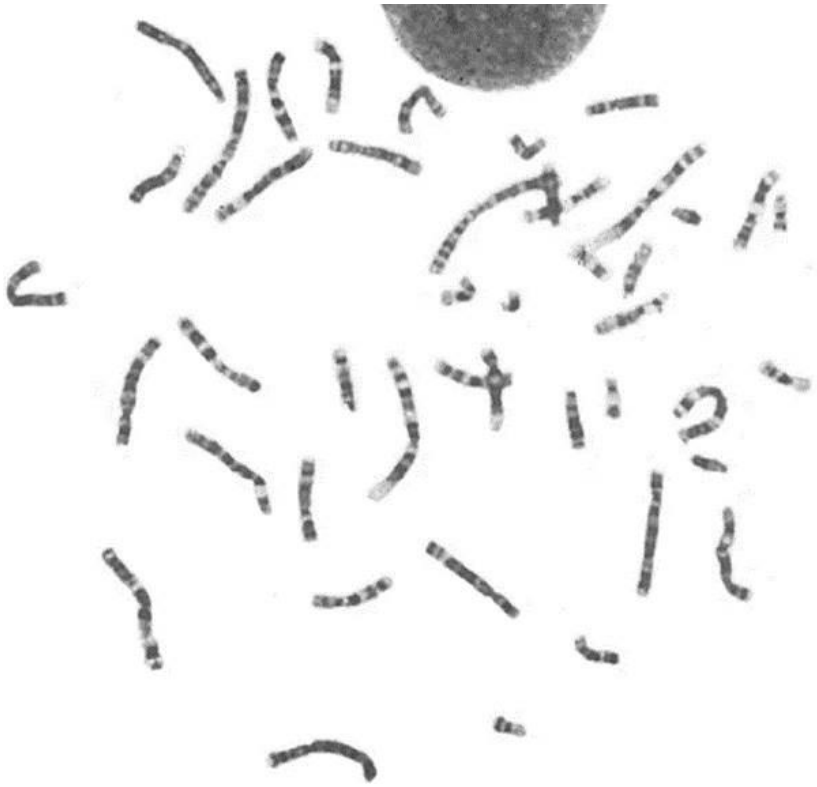

Figure 07: Chromosomes visualized under microscope

Consequently, after performing any type of molecular techniques of cytogenetics, we obtain what we call "a Karyotype", a statement that gives the total number of chromosomes and the sex-chromosome constitution (for example: 46, XX), together with the Karyogram that represents an image showing an individual's complete set of chromosomes displayed as homologous pairs, meaning that, it is a laboratory-produced picture of a person's chromosomes isolated from an individual cell and arranged in numerical order. Looking at it, we can tell whether or not somebody has all the proper number of chromosomes, and that way we can look at the X and the Y chromosomes and determine if it's a female **(fig. 8)** or a male **(fig. 9)**.

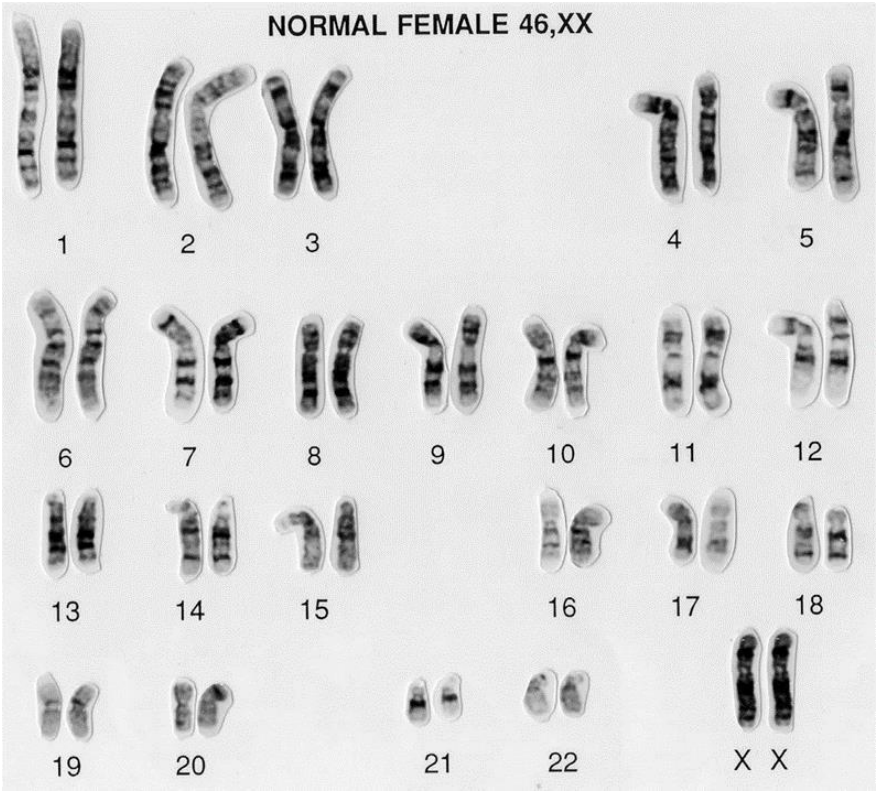

Figure 08: Normal female 46, XX human karyotype

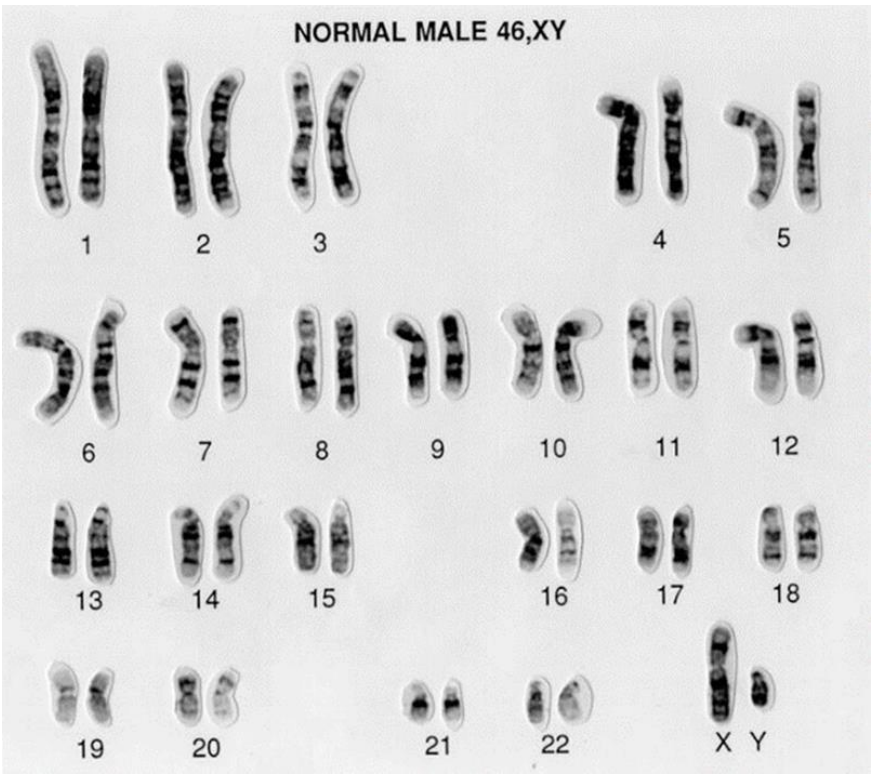

Figure 09: Normal male 46, XY human karyotype

Somebody might order a chromosome study and look at a karyotype if they were worried that a child might have an extra or missing bit of chromosome material. So, one of the most common things we can see on karyotyping is an extra Chromosome 21, which is associated with Down syndrome (DS). We also get karyotypes when pregnant women choose to have testing on their unborn fetus, and the karyotype allows the providers to look at and count the chromosomes to determine whether or not the child is affected by having an extra chromosome [2].

I.5. Chromosomal aberrations

Chromosomal aberrations (CAs) or chromosomal abnormalities are alterations at the chromosomal level that consist of the presence or absence of entire chromosomes or the breakage of existing ones.

Even though there are many studies and technological advances, little is known about the mechanisms associated with the process of induced lesions in DNA and how these might lead to CAs. However, such changes may indicate changes in cellular homeostasis, which are critical in genomic instability and crucial to understand the processes of mutagenesis and carcinogenesis **(Guimaraes et al., 2014)**.

I.5.1. Causes of CAs

Any chemical or physical agent capable of triggering chromosomal alteration is called clastogenic agent.

The clastogenicity may come as a consequence of the direct interaction of these agents with the DNA or the indirect processes which interfere with the replication and repair of the molecule. The chemical agents are the ones that induce chromosomal damage including alkylating agents, intercalators, DNA repair inhibitors, and other substances, while the physical agents can be represented by radiations.

Likewise, a CA can occur when a child inherits too many or too few chromosomes, either because of the age of the mother or because the gametes do not divide evenly when they are forming. Therefore, some cells have more than 46 chromosomes.

I.5.2. Types of CAs

DNA damage, formed intuitively or induced by miscellaneous chemical and physical agents, or during the process of DNA repair, can be repaired or processed, but many of them can lead to the formation of numerical and structural CAs.

The breaks in DNA produced by mutagens may involve single-strand breaks (SSB) and double-strand breaks (DSB). These latter could be the main damage resulting directly in various types of CA, which can be detected in the first mitotic division, subsequent to the exposure of the cells to the mutagenic compound.

1.5.2.1. Numerical abnormalities

Numerical abnormalities are defects known when there is a different number of chromosomes in the cells of the body from what is usually found, it is usually called Aneuploidy, and is the opposite of Euploidy which refers to having complete chromosome sets.

a) Trisomy

Trisomies refer to the presence of an additional chromosome rather than the usual pair of chromosomes.

For example, trisomy 21 that means the presence of 3 copies of chromosome 21, which results in DS, and trisomy 18 which causes Edwards syndrome and trisomy 13, also known as Patau syndrome. The presence of this extra copy of the chromosome is life-threatening and may lead to severe intellectual disabilities and terrible physical abnormalities.

They are generally detected during pregnancy by prenatal testing.

If the defect touches a sex-chromosome, we get a genetic disorder known as Klinefelter syndrome that affects males born with an extra copy of the X chromosome [3] **(fig. 10)**.

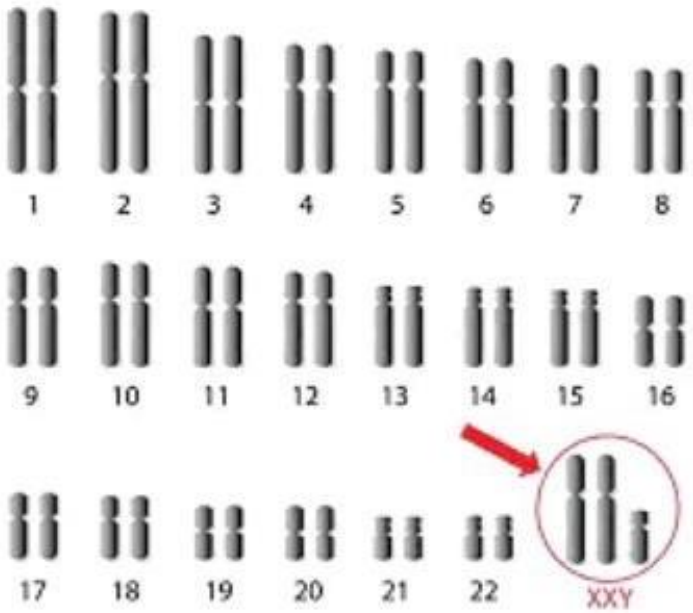

Figure 10: Chromosomal pattern in Klinefelter Syndrome

b) Monosomy

It is the absence of one copy of a pair of chromosomes. Therefore, there is a total of 45 chromosomes in each cell of the body, rather than 46.

It may happen that the abnormality touches a sex-chromosome (monosomy of sex chromosome (2n-1)), like the presence of a single X chromosome and the absence of the other one or it may be partially missing, in a female. Thus, she gets Turner syndrome **(fig. 11)**. So, her karyotype would be XO instead of XX.

Turner syndrome can be discovered prenatally, during childhood, or in early adolescence.

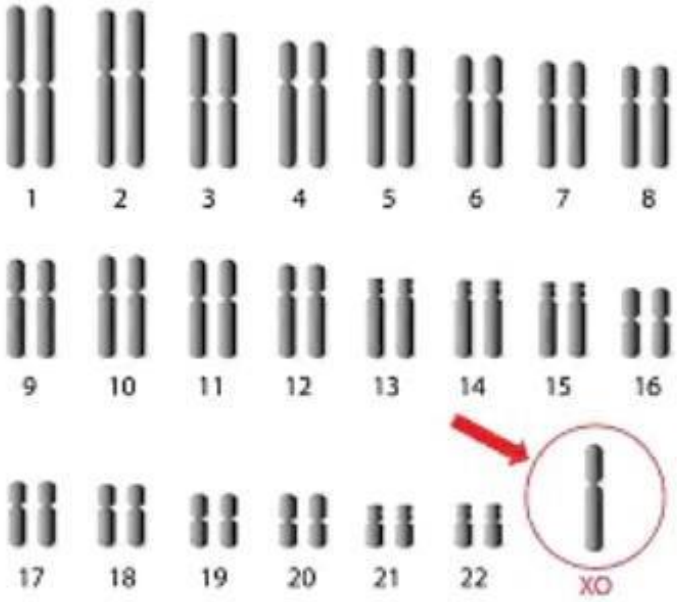

Figure 11: Chromosomal pattern in Turner Syndrome

c) Triploidy and Tetraploidy

Triploidy is the presence of an additional set of chromosomes in the cell for a total of 69 chromosomes rather than the normal 46 chromosomes per cell. The extra set of chromosomes originates either from the father or the mother during fertilization. It is written as 3n rather than 2n, while tetraploidy is 4n. Babies with tetraploidy have four copies of each chromosome, for a total of 92 chromosomes.

Tetraploidy is an infrequent chromosomal abnormality, but triploidy occurs fairly often.

1.5.2.2. Structural abnormalities

Structural abnormalities occur when part of a chromosome is missing, extra, switched to another chromosome, or turned upside down. They can take place accidentally during egg or sperm formation or fetal development stages **(Strachan and Read, 2018)**.

An altered chromosome structure may take several different forms, and result in various disorders or malignancies:

a) Deletion (del)

It is a missing or deleted portion of the chromosome. (Some famous disorders in humans with deletions are: Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder).

b) Duplication (dup)

A duplicated portion of the chromosome resulting in extra genetic material. (Known human disorders with duplications include Charcot-Marie-Tooth disease type 1A, which may be induced by duplication of the gene that encodes for peripheral myelin protein 22 (PMP22) on chromosome 17).

c) Inversion (inv)

A portion of the chromosome can be broken off and turned upside down and reattached. As a consequence, the genetic material will be inverted.

d) Insertion (ins)

It is the case of when a portion of one chromosome is deleted from its normal place and integrated into another chromosome.

e) Translocation (t)

There are two types included:

- Reciprocal translocation: where two nonhomologous chromosomes exchange segments **(fig. 12.A)**.
- Robertsonian translocation: a special type of translocation that joins two acrocentric chromosomes (N° 13, 14, 15, 21, and 22). The short arm of each of these chromosomes is very small and comprises very similar DNA **(fig. 12.B)**.

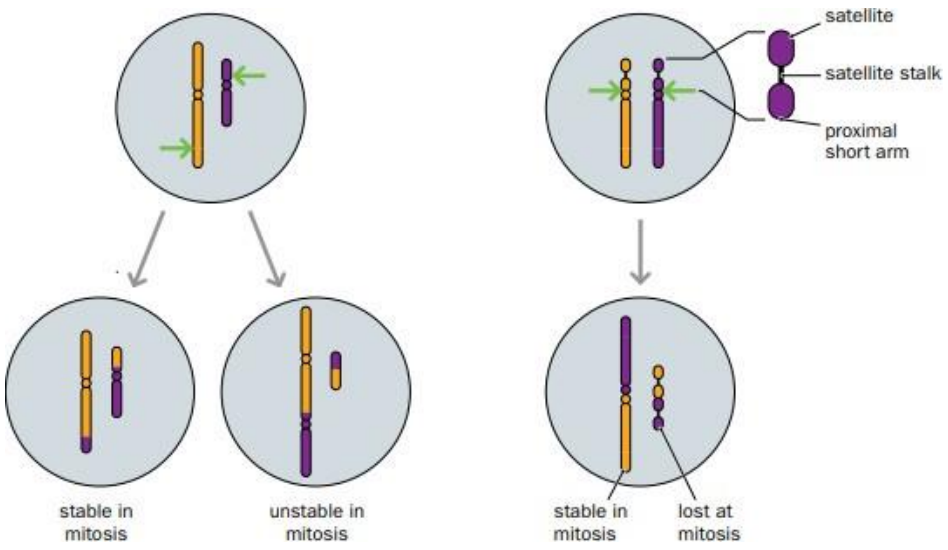

Figure 12: A. Reciprocal translocation and **B.** Robertsonian translocation

f) Ring

A portion of a chromosome can be broken off and form a circle called ring chromosome. This can happen with or without loss of genetic material. Usually comes from DSB at ends or telomere dysfunction **(fig. 13)**.

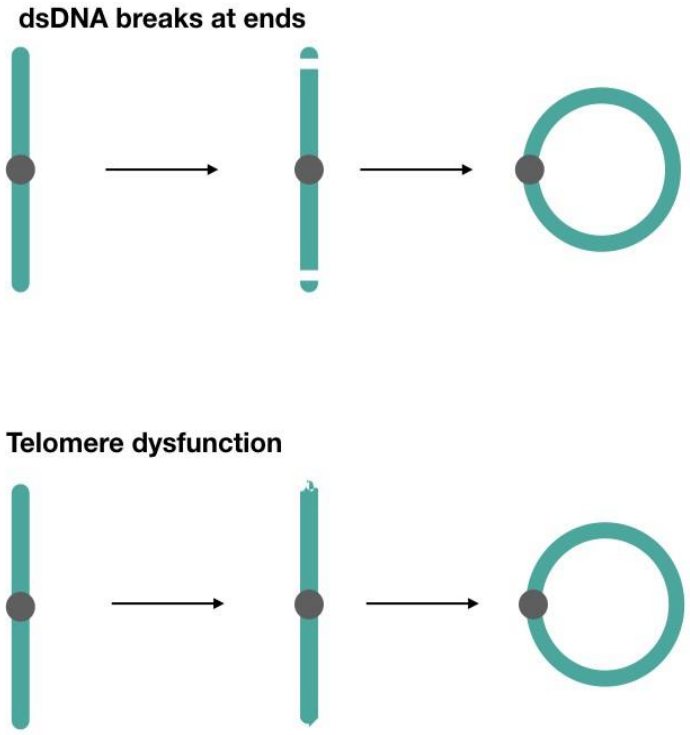

Figure 13: Formation of ring chromosome

g) Isochromosome (I)

It occurs when the arms of the chromosome are mirror images of each other **(fig. 14)**.

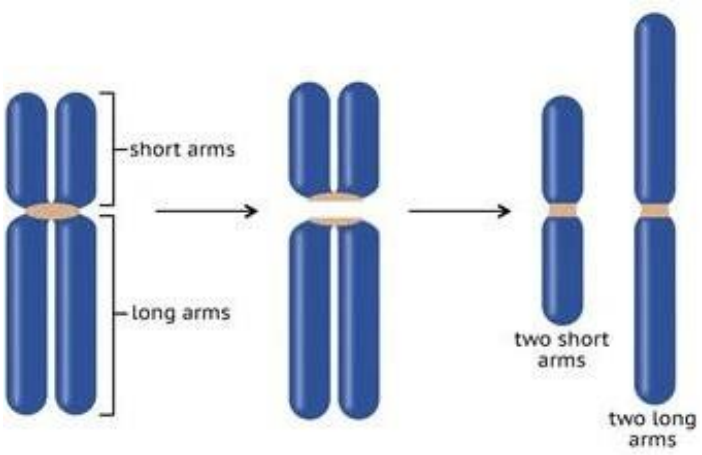

Figure 14: Formation of an Isochromosome

I.5.3. Chromosome Banding Techniques

Observing chromosomes and detecting genetic aberrations relies on the average resolution used which also depends on different elements, such as the optical characteristics of the microscope, the complex manner in which the DNA is packaged into chromosomes and the quality of the metaphase preparations. Studying human chromosomes is easier for mitosis than meiosis. This later can be studied only in testicular or ovarian samples. While for mitosis, we can use samples from the bone marrow, from where we get non-dividing cells and then we propagate them in cell culture under laboratory conditions, or from the circulating blood cells and skin fibroblasts as people rarely minds giving a small blood sample, and the T lymphocytes in blood can be easily induced to divide by treatment with lectins (such as phytohemagglutinin) [4].

1.5.3.1. Standard chromosome banding techniques

a) G-banding

The chromosomes are subjected to controlled digestion with trypsin before being stained with Giemsa, a DNA-binding chemical dye. Positively staining dark bands are known as G bands. Pale bands are G negative **(fig. 15.A)**.

b) Q-banding

The chromosomes are stained with a fluorescent dye that binds preferentially to AT-rich DNA, notably quinacrine or DAPI (4', 6-diamidino-2-phenylindole), and viewed by ultraviolet fluorescence. Fluorescing bands are called Q bands and mark the same chromosomal segments as G bands **(fig. 15.B)**.

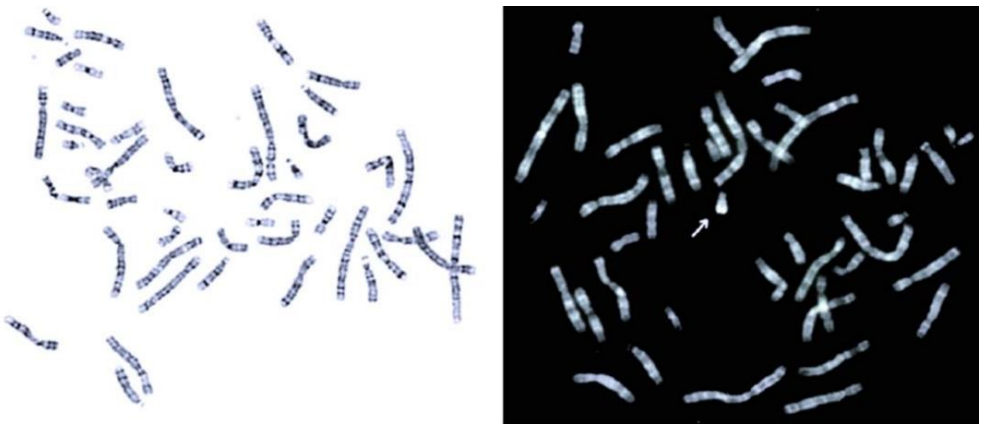

Figure 15: A. G-banding and **B.** Q-banding

c) R-banding

The reverse of the G-banding pattern. The chromosomes are heat-denatured in saline before being stained with Giemsa. The heat treatment denatures AT-rich DNA, and R bands are Q negative. As **(fig. 16)** shows, the light and dark bands are the opposite of those obtained with G-banding.

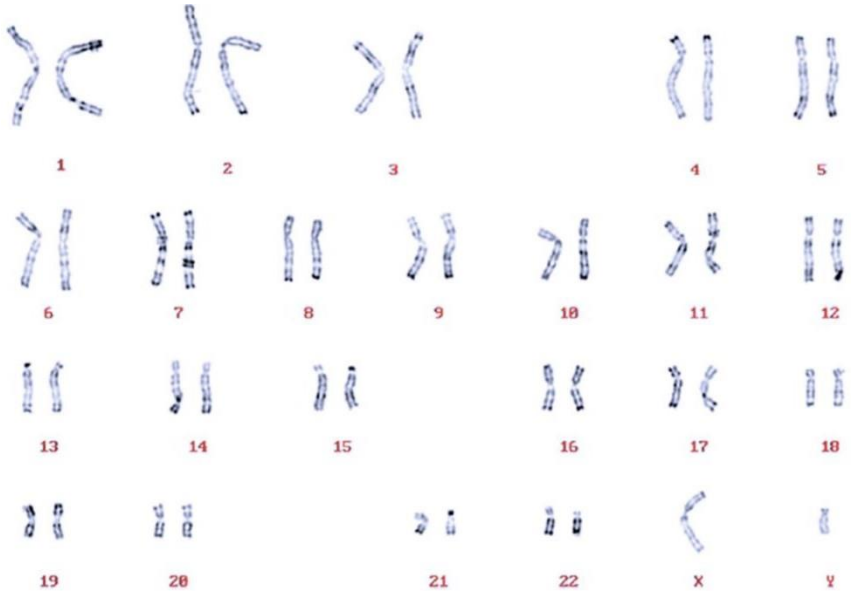

Figure 16: R-banding (reverse banding)

d) T-banding

Identifies a subset of the R bands that are especially concentrated close to the telomeres.

e) C-banding

This is thought to demonstrate constitutive heterochromatin, mainly at the centromeres.

Besides the above, there are other techniques for chromosome banding, like H-banding and N-banding.

1.5.3.2. Hybridization techniques

For higher resolution analyses, specific DNA sequences within chromosomes need to be detected, using a variety of techniques that have been developed with the emergence of the Genomics, as FISH and CGH techniques (that will be briefly explained), Spectral Karyotyping (SKY), Chromosome Conformation Capture technique (3C) and its varieties (4C, 5C, Hi-C), New Generation Sequencing (NGS), etc.,

a) FISH

Fluorescence *in situ* Hybridization (abbreviated FISH) is a laboratory approach used to determine and detect a specific DNA sequence on a chromosome.

In this technique, the full set of chromosomes is fixed to a glass slide and further exposed to a "probe" (a small piece of purified DNA tagged with a fluorescent color).

The fluorescently labeled probe finds and also binds to its matching sequence within the set of chromosomes **(fig. 17)**. With the use of a special microscope, the chromosome and sub-chromosomal position where the fluorescent probe bound can be observed. FISH probes can hybridize to metaphase spreads and also to interphase chromosomes **(fig. 18)**, allowing a much higher resolution than G-banding karyotyping [1].

FISH analysis has been developed through the years and evolved into even more advanced methods to analyze chromosome and gene modifications, for example: Two-color FISH, Multiplex FISH (M-FISH), a 24-color karyotyping technique, Chromosome Orientation FISH (CO-FISH), Fiber FISH, Three-dimensional FISH (3D-FISH).

This method can be used to identify deletions or duplications that are too small to be detected by conventional methods. A variety of syndromes that are difficult to identify by conventional methods are identified by FISH. In leukemias and other neoplasias, where specific chromosome

rearrangements correlate with the type and severity of cancer; other probes have been particularly valuable.

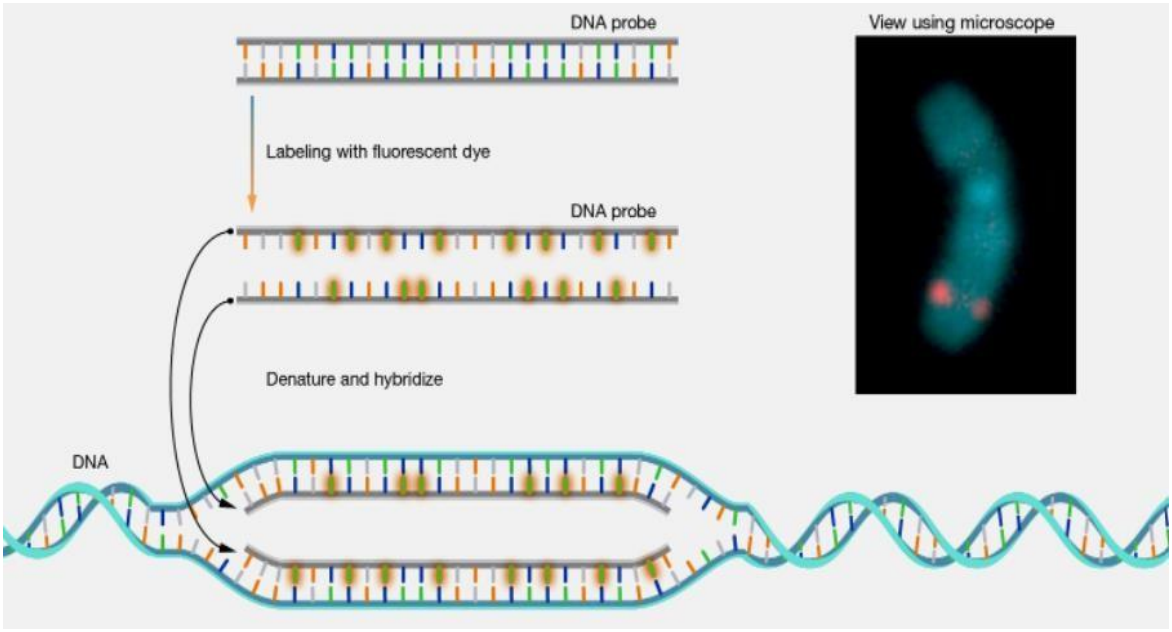

Figure 17: Illustration of the basic FISH approach

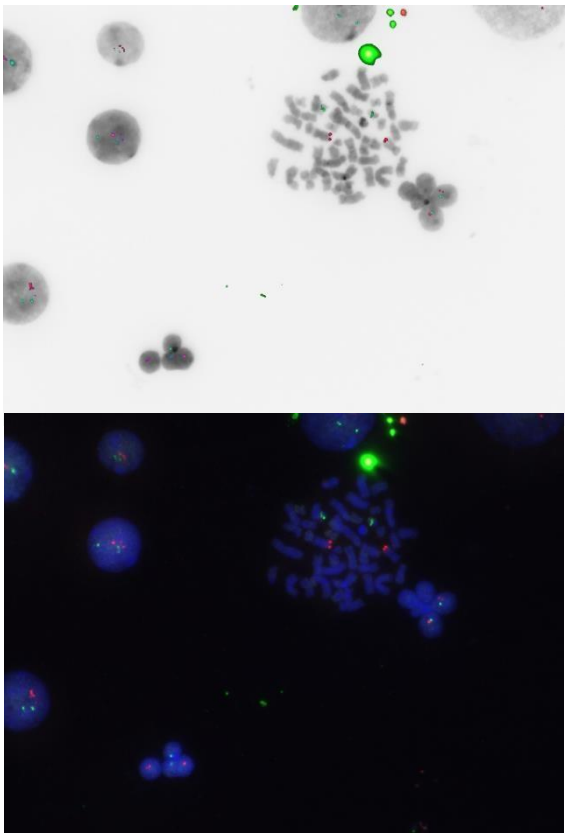

Figure 18: FISH analysis of chromosomes in metaphase

b) CGH

Comparative Genomic Hybridization (CGH) is a molecular cytogenetic method, used for analyzing copy number variations (CNVs) relative to ploidy level in the DNA of a test sample compared to a reference sample, without the requirement of cell culture.

The purpose of this method is to quickly and effectively compare two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or sub-chromosomal regions (a portion of a whole chromosome).

This technique was originally developed for the evaluation of the differences between the chromosomal complements of solid tumor and normal tissue, and has an improved resolution of 5–10 megabases compared to the more traditional cytogenetic analysis techniques which are limited by the resolution of the microscope utilized. Recently, CGH technique progressed into array-based CGH (aCGH) which allows the identification of gene or sequence deletions and amplifications in a very precise manner. In one single experiment, many thousand sequences are analyzed simultaneously **(fig. 19)**.

Yet, based on its technical limitations, there is little reason to apply CGH to chromosomes of metaphase cells in routine diagnostic settings, because it only detects unbalanced chromosomal abnormalities.

Although it has contributed to the current knowledge of genomic alterations in hematologic malignancies, more elaborated CGH procedures like specific matrix-CGH DNA chips have to be applied to meet the clinical needs, and to overcome most of the limitations, which results in the opening of new approaches for diagnosis and identification of genetically defined leukemia and lymphoma subgroups **(Lichter et al., 2000)**.

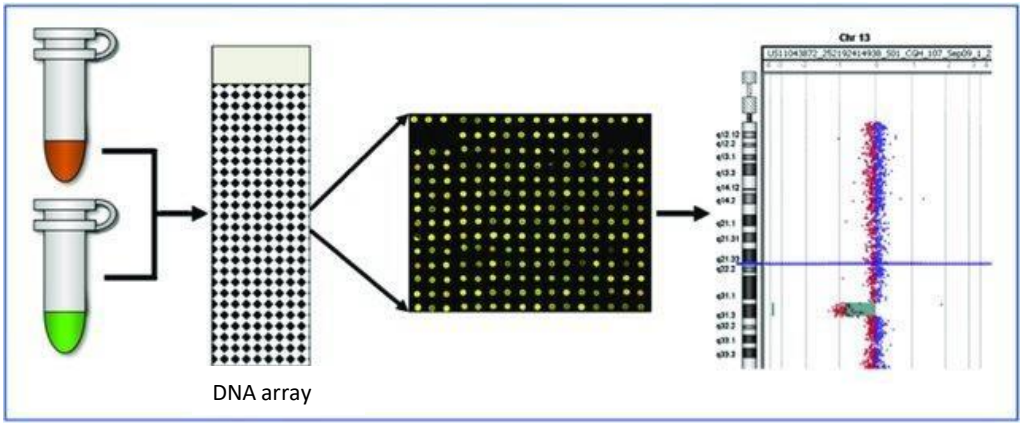

Figure 19: array-based CGH technique [5]

Chapter 2

Recurrent diseases induced by Chromosomal aberrations

II.1. Introduction to Oncology and Hematology

Genomic instability can be seen at different levels: chromosomal losses, gains, and rearrangements, structural variants, point mutations, and epigenetic dysregulation. It is the normal feature of tumor cells that evolute gradually after chromosomal rearrangements which often create novel chimeric genes implicated in cancer, known as oncogenes **(Strachan & Read, 2018).**

As all the cells of the body have a tightly regulated system that controls their growth, maturity, reproduction and eventual death, cancer starts when part of these cells goes out-of-control and grows abnormally. Here it appears the Oncology, which is the medical specialty responsible for preventing, diagnosing, staging, and treating cancer. It firmly depends on diagnostic tools which differ and then, provide subspecialties of oncology:

- Medical Oncology that treats cancer with different therapeutic interventions such as chemotherapy, hormone therapy and immunotherapy.
- Radiation Oncology that uses focused, high-energy radiation to destroy cancer spreading cells.
- Surgical Oncology that approaches the treatment of cancer through surgical intervention.

Common methods include blood tests for biological or tumor markers. Rise of these markers in blood may be the indication of the presence of the cancer.

As the blood plays important roles in human health, from the transport of vital substances such as oxygen and nutrients around the body, helping to fight off disease, problems with this vital fluid can have an effect on the body's systems like the lymphatic system, a network of tissues and organs that sweeps away wastes.

Sometimes, blood disorders arise from problems with the bone marrow, the major producer of blood cells.

In consequence, Hematology, a subspecialty of internal medicine, came to understand how these problems occur, how they affect a person's health and how to treat them **(Wan, 2014).**

For a better understanding, we need to have an overview on blood and immune system cells.

Hematopoiesis is the process through which the body produces all of the cellular components of blood and blood plasma. It occurs within the hematopoietic system, which includes organs and tissues such as the bone marrow, liver, and spleen.

It begins during the first weeks of embryonic development (primitive hematopoiesis), and continues for the life of an individual.

The figure below shows the development of different blood cells from hematopoietic stem cells (HSC) to mature cells **(fig. 20)** [6].

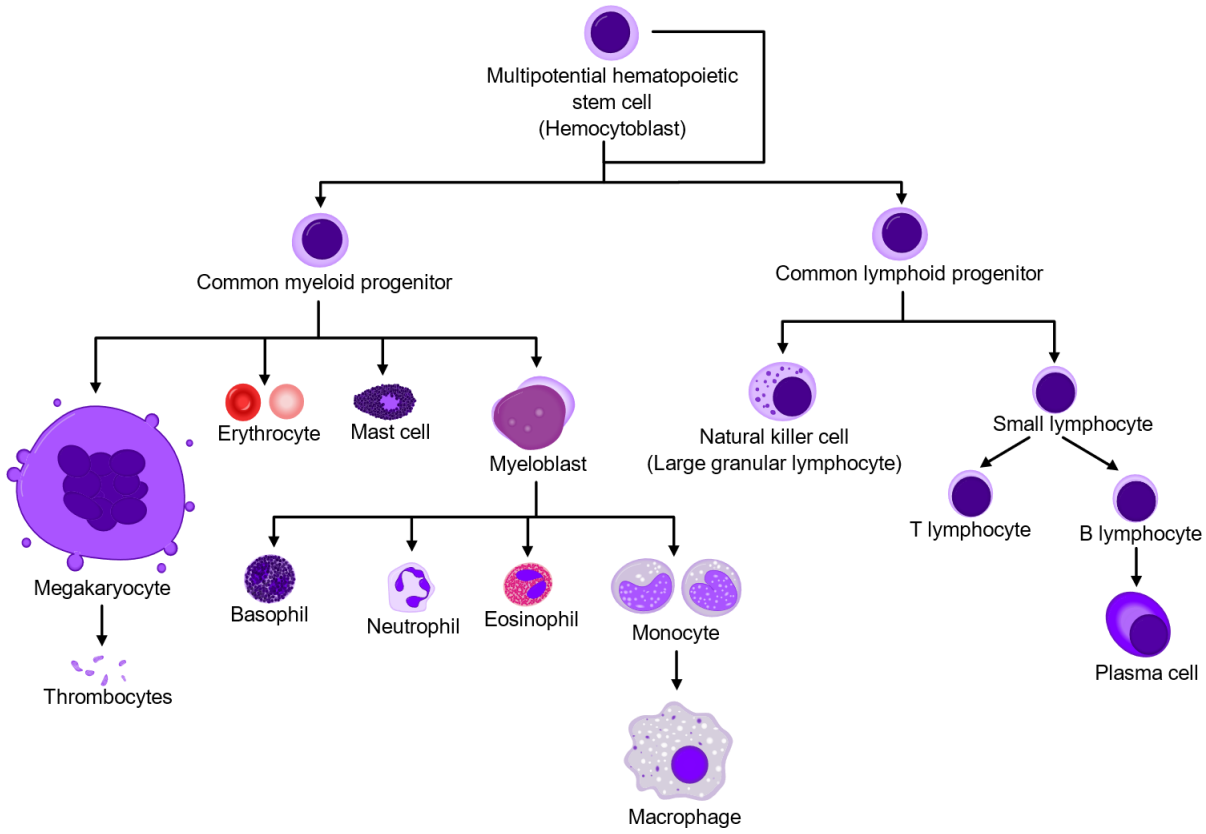

Figure 20: Simplified Hematopoiesis in human

In this chapter, we will turn our attention to a few blood cell diseases, such as leukemias, lymphomas and myelomas, and reveal the chromosomal abnormalities involved.

Therefore, detecting the root causes of these CAs, which then lead to life-threatening pathologies, is a great step towards finding an accurate prognosis of these disorders, thus, successfully curing them.

II.2. Leukemia/ Lymphoma

Leukemia, also spelled Leukaemia, is a type of blood cancers that usually begin in the bone marrow and result in high numbers of abnormal blood cells. These blood cells are not fully developed and are called blasts or leukemia cells.

It usually affects the leukocytes, or white blood cells (WBCs). As more of these cancer cells are being produced, they begin to overcrowd the blood, preventing the healthy WBCs from growing and functioning normally.

Leukemia develops when the DNA of developing blood cells, mainly WBCs, sustains damage. This causes the blood cells to grow and divide uncontrollably

There are four main types of leukemia:

- Acute
- Chronic
- Lymphocytic
- Myelogenous

Both lymphoma and leukemia describe cancers that derive from blood-borne cells. If the disease produces tissue masses (tumors) in the lymph nodes, or extra-nodal sites, so it is called a lymphoma. While the diseases found only in circulating blood is a leukemia.

II.2.1. Acute Lymphoblastic Leukemia (ALL)

ALL is a rare hematologic disorder manifesting as malignant transformation and proliferation of immature lymphoid progenitor cells in the bone marrow, peripheral blood and extramedullary sites. While 80% of ALL occurs in children, it is a devastating disease when it occurs in adults. ALL results in death within months if left untreated. Overall survival has improved over the last decade; also, with the introduction of new targeted therapies. While dose-intensification strategies have led to a significant improvement in outcomes for pediatric patients, prognosis for the elderly remains very poor.

And despite the high rate of response to induction chemotherapy, only 30–40% of adult patients with ALL will achieve long-term remission.

To examine ALL, most cytogeneticists should be able to confront its several technical challenges, including frequent poor chromosome morphology, low mitotic index, and samples that have a marked tendency to clot during harvest **(Wan, 2014).**

ALL is a heterogeneously disease, and patients should be treated in international study group protocols. Its prognosis is determined by immunophenotype, cytogenetics and molecular markers which influences therapeutic approaches.

According to the World Health Organization (WHO), ALL is classified in two main categories, B-cell lymphoblastic leukemia and T-cell lymphoblastic leukemia **(Table 01).**

Table 01: WHO Classification of ALL (a comprehensive review and 2017 update)

B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
B-cell lymphoblastic leukemia/lymphoma, with recurrent genetic abnormalities
- B-cell lymphoblastic leukemia/lymphoma with hypodiploidy
- B-cell lymphoblastic leukemia/lymphoma with hyperdiploidy
- B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2)[BCR-ABL1]
- B-cell lymphoblastic leukemia/lymphoma with t(v;11q23)[MLL rearranged]
- B-cell lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22)[ETV6-RUNX1]
- B-cell lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3)[TCF3-PBX1]
- B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32)[IL3-IGH]
- B-cell lymphoblastic leukemia/lymphoma with intrachromosomal amplification of chromosome 21 (iAMP21)
- B-cell lymphoblastic leukemia/lymphoma with translocations involving tyrosine kinases or cytokine receptors (‘BCR-ABL1–like ALL’)
T-cell lymphoblastic leukemia/lymphomas
- Early T-cell precursor lymphoblastic leukemia

As shown above, when ALL develops from B cells; it is called:

B-cell acute lymphoblastic leukemia (B-Cell ALL).

About 75 percent of ALL cases are the B-cell type, and the rest are T-cell type.

In the case of B-cell ALL, gene changes cause immature B cells to grow very quickly. These cells take over the bone marrow and then spill over into the blood. They can also collect in tissues like the spleen, thymus, and central nervous system (brain and spinal cord).

II.2.1.1. Common recurring cytogenetic abnormalities in ALL and prognosis

-t(9;22): one of the most common gene changes is a balanced translocation of the Abelson gene (ABL1) in chromosome 9q34 with the breakpoint cluster region (BCR) gene in chromosome 22q11.2, and then, the generation of a [BCR-ABL1] fusion oncogene, which in turn translates into a BCR-ABL1 oncoprotein that has a high tyrosine kinase activity, so it induces a disorder called **Chronic Myelogenous Leukemia (CML) (fig. 21)**.

The term "chronic" in CML or even in **CLL** (that stands for **Chronic Lymphocytic Leukemia**; another type of cancer in which the bone marrow makes too many lymphocytes, represented in del(13q), tris 12 with a favorable prognosis or with del(17p), del(11q) with an unfavorable prognosis) comes from the fact that this leukemia typically progresses more slowly than other types of leukemia.

(9;22) translocation was the first CA discovered in cancer using the cytogenetics technique in 1960. Then, in 1973, as chromosome preparation techniques improved, **Janet Rowley** demonstrated that the Philadelphia chromosome was the result of the mentioned translocation. That being said, the Philadelphia Chromosome is present in both ALL and CML diseases **(fig. 22)**.

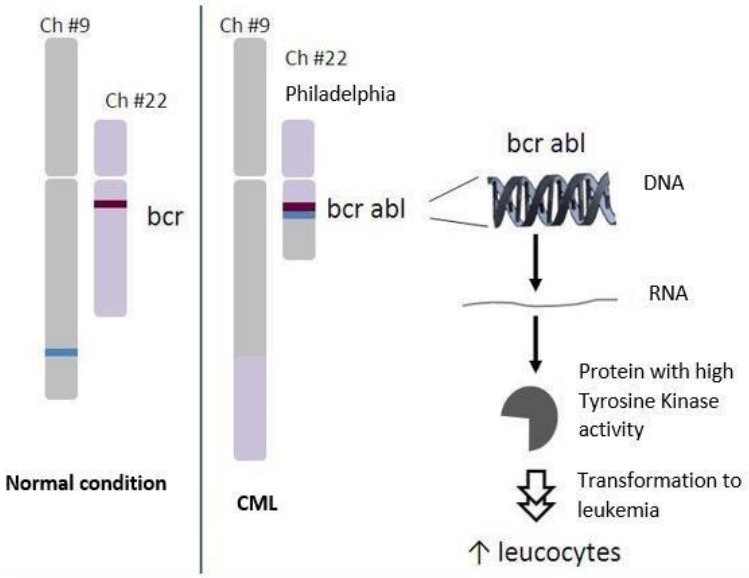

Figure 21: The translocation t(9;22) (q34;q11.2) in CML

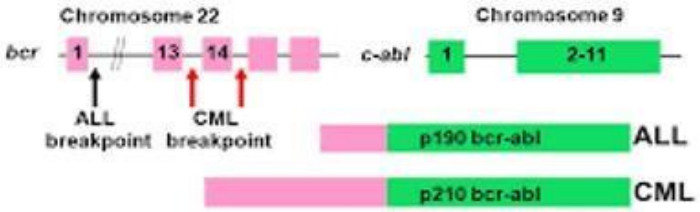

Figure 22: BCR-ABL1 gene and fusion protein tyrosine kinase

-t(1;19): another frequent translocation in B-Cell ALL is the translocation that occurs between chromosome 1 and 19. It is observed in both adult and pediatric populations at an overall frequency of 6%. This translocation can occur in a balanced – t (1;19)(q23;p13) – or unbalanced – der(19)t(1;19)(q23;p13) – form and can result in the fusion of TCF3 (transcription factor 3) found at 19p13 and PBX1 (pre-B cell leukemia homeobox 1) found at 1q23 to form a chimeric gene whose protein product changes cell differentiation arrest, among other cellular processes **(fig. 23) (Tirado et al., 2015)**.

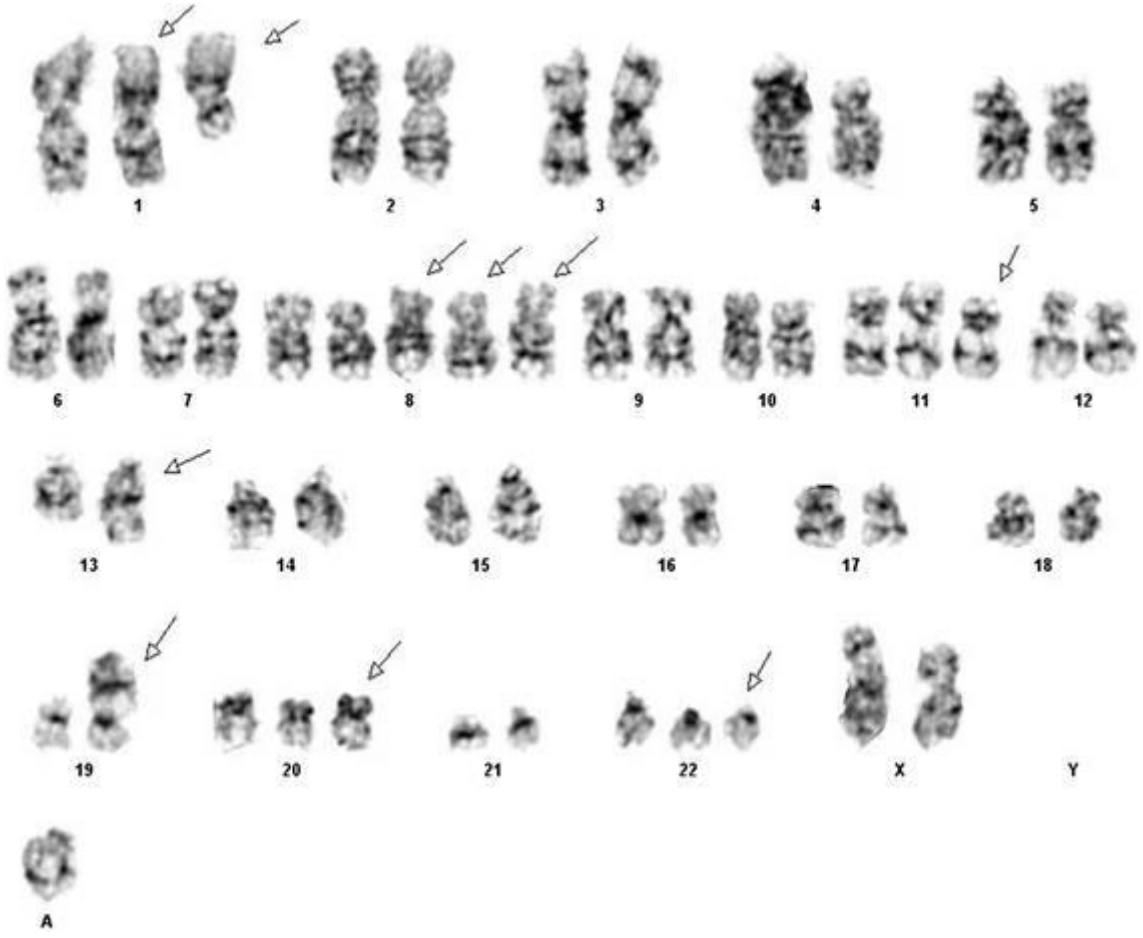

Figure 23: Karyotype of female patient revealing t(1;19) in a hyperdiploid context

-t(12;21): the very common chromosomal translocation that occurs in pediatric leukemia. It is associated with favorable prognosis **(table 02)**. It results from the break and rearrangement of chromosomes 12p13 and 21q22, leading to the fusion of two genes. [ETV6-RUNX1] rearrangement results in the in-frame fusion of the 5′-region DNA-binding domain of ETV6 to almost the entire RUNX1 locus **(fig. 24) (Jin et al., 2016)**.

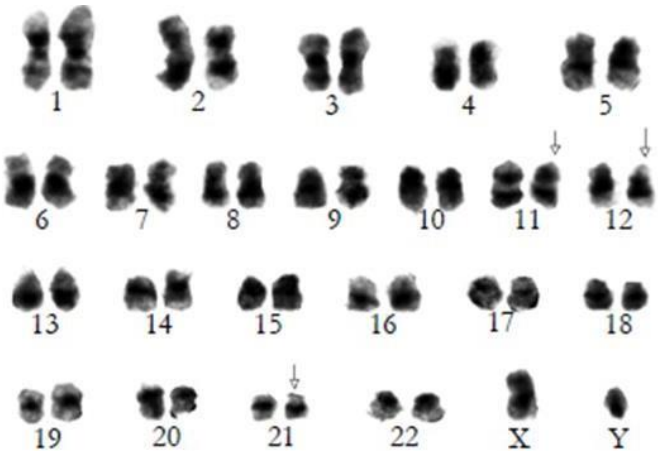

Figure 24: Variant Translocation of ETV6 and RUNX1 in a Case of Pediatric ALL

Table 02: Prognostic risk of B-Cell ALL

Anomaly	**Prognosis**
t(12;21)(p13;q22) ETV6-RUNX1	Favorable
Hyperdiploidy (# 4, 6, 10, 17, 18, 21)	Favorable
t(9;22)(q34;q11) BCR-ABL1	Unfavorable
t(4;11)(q21;q23) AFF1/MLL	Unfavorable
iAMP21 amp RUNX1	Unfavorable
t(17 ;19)(q22 ;p13) HLF-TCF3	Unfavorable
Hyperdiploidy (# 1, 3, 7, 11, 17)	Unfavorable
MLL rearrangement 11q23	Variable

In addition, B-Cell lymphoma encompasses a biologically and clinically diverse set of disease subtypes, many of which are difficult to separate from one another.

Diffuse Large B-Cell Lymphoma (DLBCL) is one of these subtypes, it represents a cancer that starts in WBCs as well.

It usually grows fast in lymph nodes, but 3 out of 4 people are disease-free after treatment, and about half are cured. It does not have very known causes but, in general, it affects middle-aged or older men (on average, people are diagnosed with DLBCL at 64 years old), especially those who got exposed to some types of radiation.

Immune pancytopenia is another common disorder that can develop in a patient with DLBCL after he gets treated with chemotherapy. It is a medical condition in which there is significant reduction in the number of red and white blood cells, as well as platelets.

II.2.1.2. Risk factors

Even though it is usually not so clear what causes the gene mutations implicated in the different cases of ALL; However, there is a range of risk factors for leukemia that researchers have identified and which can increase a person's chances of having gene changes that lead to B-cell ALL:

- **Certain genetic disorders:** such as Down syndrome, Ataxia telangiectasia, Fanconi anemia, Klinefelter syndrome…etc.
- **Environmental factors:** high levels of radiation can sometimes cause ALL many years later. In addition, exposure to some agricultural chemicals such as pesticides, fuels and other toxic chemicals is a prominent factor that provokes cells to develop into cancer cells. According to a geographic study on cancer mortality and occurrence of congenital malformations in Campania, a Region of Southern Italy which is characterized by multiple toxic dumping sites, the regional population suffers from health problems such as lung cancer, leukemia and gastric cancer in both sexes, breast cancer in women, childhood leukemia, perinatal mortality and birth defects **(Comba et al., 2006)**.
- **Artificial ionizing radiation and chemotherapy:** people who received chemotherapy treatment for a previous cancer have a higher risk to develop leukemia later in life.
- **Exposure to benzene:** this is a solvent that manufacturers use in some cleaning products and hair dyes.
- **Certain viruses:** the human T-lymphotropic virus (HTLV-1) has links to leukemia. Additionally, people with Epstein-Barr virus (EBV) infections may be more likely to develop certain kinds of B-cell ALL.

II.2.2. Acute Myeloid Leukemia (AML)

Abnormal differentiation of myeloid cells results in a high level of immature malignant cells and fewer differentiated red blood cells (RBCs), platelets and WBCs. So, AML is a disorder characterized by a clonal proliferation derived from primitive hematopoietic stem cells or progenitor cells. (In cancer cytogenetics, an abnormal cytogenetic clone is defined as a population of cells with the same chromosome complement that is derived from a single progenitor. A clone must contain at least two cells with the same abnormality if it is a chromosome gain or a structural rearrangement. If the abnormality is loss of a chromosome, the same loss must be accounted in at least three cells to be accepted as clonal **(Heerema, 2017)**.

AML starts in the bone marrow but most often it quickly progresses and moves into the blood, as well **(fig. 25)**. It can sometimes spread to other parts of the body including the lymph nodes, liver, spleen, central nervous system (brain and spinal cord), and testicles, but without forming tumors. Therefore, AML is not staged like most other cancers.

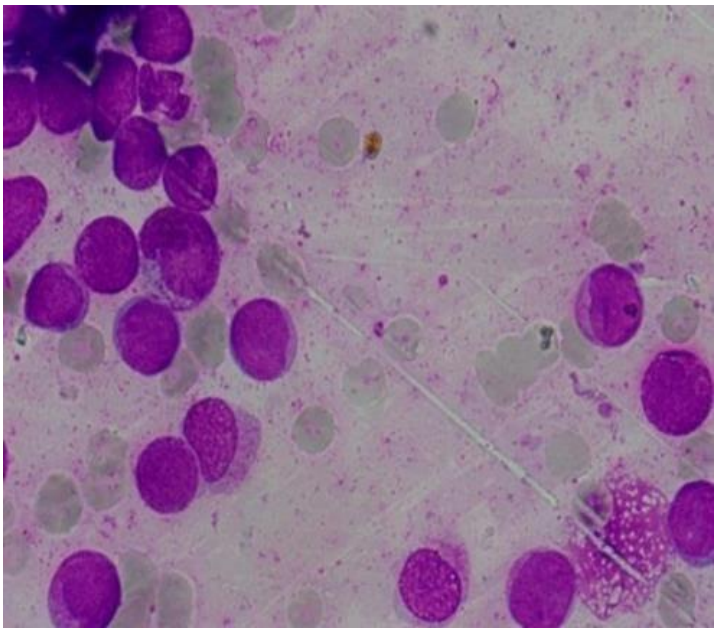

Figure 25: Photomicrograph of AML with Leishman stain, 1000X [7]

II.2.2.1. Classification of AML

AML is generally classified based on either French-American-British (FAB) or World Health Organization (WHO) systems.

A. The French-American-British (FAB) classification of AML

In the 1970s, a group of French, American, and British leukemia experts divided AML into subtypes, from M0 to M7, based on the cell type the leukemia developed from and how mature the cells are **(table 03)**.

Table 03: FAB classification of AML (1970s' classification)

FAB subtype	Name
M0	Undifferentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M4 eos	Acute myelomonocytic leukemia with eosinophilia
M5	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryoblastic leukemia

From the subtype M0 to the subtype M5, they all start in immature forms of WBCs. However, M6 AML starts in very immature forms of red blood cells, while M7 AML starts in immature forms of cells that make platelets [6] .

B. World Health Organization (WHO) classification of AML

The WHO system tried, most recently, to include factors that are now known to affect prognosis in the classification of AML, it divides AML into several groups [6] **(table 04)**:

Table 04: WHO classification of AML (updated in 2016)

AML group	Examples
AML with certain genetic abnormalities	- AML with a translocation between chromosomes 8 and 21 [t(8;21)] - AML with a translocation between chromosomes 9 and 11 [t(9;11)] - AML (megakaryoblastic) with a translocation between chromosomes 1 and 22 [t(1:22)]
AML with myelodysplasia-related changes (MRC)	- Patients with a previous history of a myelodysplastic syndrome (MDS) or a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) - An MDS-related cytogenetic abnormality
AML related to previous chemotherapy or radiation	- Therapy-related AML
AML not otherwise specified (NOS) (similar to the FAB classification)	- AML with minimal differentiation (FAB M0) - Acute megakaryoblastic leukemia (FAB M7) - Acute panmyelosis with fibrosis
Myeloid sarcoma (also known as granulocytic sarcoma or chloroma)	- Tumor of myeloblasts
Myeloid proliferations related to Down syndrome	- (ML-DS)
Undifferentiated and biphenotypic acute leukemias, also known as mixed phenotype acute leukemias (MPALs).	They are not strictly AML, but are leukemias that have both lymphocytic and myeloid features.

II.2.2.2. Prognostic factors for AML

AML cells can have different kinds of CAs, some of which can affect a person's prognosis.

We can summarize some of these chromosomal changes in the table below **(table 05)** but there are many others.

Table 05: Prognostic risk of AML

Favorable abnormalities	Unfavorable abnormalities
Translocation between chromosomes 8 and 21 " t(8;21)"	Translocation between chromosomes 9 and 22
Translocation or inversion of chromosome 16 "inv(16)"	Loss of a chromosome, (known as monosomy)
Translocation between chromosomes 15 and 17 "t(15;17)"	Complex changes (those involving 3 or more chromosomes)

Recurrent cases indicating a bad prognosis:

- Generally, people over 60 years old with several medical conditions.
- AML that develops after a person is treated for another cancer.
- Having a prior blood disorder such as a myelodysplastic syndrome.
- Leukemia that has spread to the area around the brain and spinal cord.

II.3. Multiple Myeloma (MM)

Multiple Myeloma or simply "myeloma" is a cancer of the bone marrow plasma cells where the antibodies that fight against infectious agents such as viruses and bacteria are made.

It is called "multiple" because there are frequently multiple areas in the bone marrow where it grows.

It is the second most common blood cancer in the world and it is more likely to occur in people of African descent.

II.3.1. Impacts of MM on the body

MM affects the places where bone marrow is active in an adult. We will find more about how the disease impacts each of the: bones, blood, kidneys, and immune system **(table 06)**.

Table 06: How MM affects important parts of the body

Part of the body	Impact on it
Bones	Myeloma cells increase bone breakdown and prevent new bone cell production, which in return, increase the risk of fractures and release the calcium in the blood, causing hypercalcemia.
Blood	Myeloma disrupts the production of RBCs and WBCs, and may cause anemia or neutropenia. This can increase risk of infection. Also, it may disrupt the platelet count, inducing thrombocytopenia which causes bleeding.
Kidneys	Up to 50% of patients with active myeloma may also develop kidney disease because of the damage linked to the toxic effects of myeloma cell-derived monoclonal proteins, hypercalcemia, or infection.
Immune system	MM suppresses the immune response as whole since it reduces the number of normal antibodies (AB), or immunoglobulins (Ig).

II.3.2. Risk factors of MM

Factors that may increase the risk of having MM include:

- **Increasing age:** most people diagnosed with MM are in their mid-60s.
- **Male sex:** Men are more likely to develop the disease than women.
- **Black race:** Black people have the tendency to develop MM more than people of other races.
- **Family history:** if MM is/was present in one member of a family, other family members are at high risk of developing the disease.

- **A connection with monoclonal gammopathy of undetermined significance (MGUS):** MM almost always starts out as MGUS. It is a condition in which an abnormal protein known as monoclonal protein or M protein is detected in the blood. It does not have clear symptoms; it can only be detected while performing blood test for another condition. Although the precise cause of MGUS is not known, genetic changes and environmental triggers appear to play a role. [2]

II.3.3. Cytogenetics in MM

Cytogenetic abnormalities are the main risk factor in MM.

- Good risk: Hyperdiploidy with 48-74 chromosomes (the extra chromosomes are the "odd numbered" chromosomes 3, 5, 7, 9, 11, 15, 19, 21).
- Intermediate risk: t(4;14)(p16.3;q32.3).
- Poor risk: del(17p) or loss of TP53, t(14;16)(q32.3;q23), t(14;20)(q32.3;q11;2), 13 monosomy/ 13q deletion , 1q21 amplification/ 1p deletion.

All these aberrations can be detected by FISH **(Heerema, 2017)**.

II.3.4. Diagnosis and treatment

Tests and procedures used to diagnose multiple myeloma include:

- Blood tests.
- Urine tests.
- Examination of your bone marrow.
- Imaging tests.

If tests indicate the presence of MM, the doctor will use the information gathered and classify the disease as stage I (a less aggressive disease) and stage 2 (the most aggressive stage) and that helps him to figure out the best treatment. Immediate treatment may not be necessary for MM that is slow growing and at an early stage.

However, if it shows signs of progression, the treatment must begin. Some of the standard treatment options may include:

- Targeted therapy.
- Immunotherapy.
- Radiation therapy.
- Bone marrow transplant.
- Corticosteroids medications.

II.4. Myelodysplastic Syndromes (MDS)

Myelodysplastic Syndromes (MDS) are a group of cancers in which immature blood cells in the bone marrow do not mature to become healthy blood cells. Sometimes, MDS are called a "bone marrow failure disorder".

Many individuals are asymptomatic, and blood cytopenia or other problems are identified as a part of a routine blood count. Meaning that, early on, no symptoms typically are marked. Later, symptoms may include chronic tiredness, shortness of breath, easy bleeding, anemia and frequent infections.

Usually, MDS affect males between 60 and 75 years more than females, yet, these diseases are rare in children **(Hosono, 2019)**.

Some types of MDS may develop into AML. According to the WHO Prognostic Scoring System (WPSS) risk groups, scientists can predict the median survival outcomes of MDS and the chance of its transformation into AML within 5 years **(fig. 26)**.

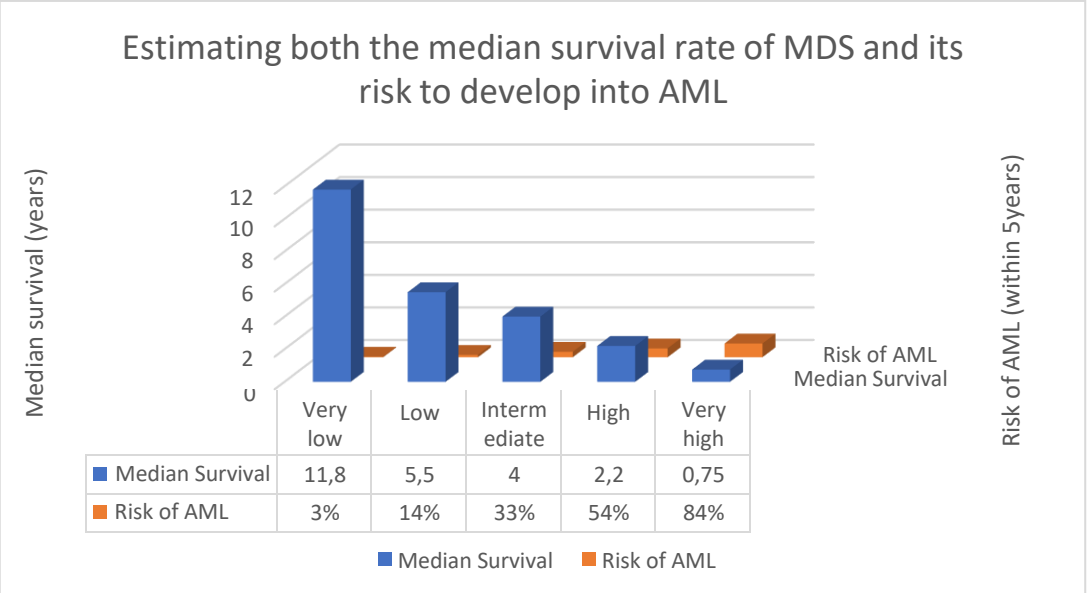

Figure 26: Estimation of outcomes: both median survival and the chance that the MDS will transform into AML within 5 years (2007's statistics)

II.4.1. Classification of MDS

MDS are classified based on both FAB and WHO systems **(table 07)**.

Table 07: Classification of MDS according to FAB and WHO systems

FAB Classification (Published in 1976 and revised in 1982)	WHO Classification (a 2008 reclassification)
- Refractory anemia - Refractory anemia with ring sideroblasts (RARS) - Refractory anemia with excess blasts (RAEB) - Refractory anemia with excess blasts in transformation (RAEB-T) - Chronic myelomonocytic leukemia (CMML)	- Refractory cytopenia - Refractory anemia with ring sideroblasts (RARS) - Refractory cytopenia with multilineage dysplasia (RCMD) - Refractory anemias with excess blasts I and II. (RAEB-I and RAEB-II) - Myelodysplasia unclassifiable - Refractory cytopenia of childhood

II.4.2. Causes and risk factors of MDS

The main causes contributing to the development of MDS may be similar to other risk factors for other types of leukemias. However, other precursors such as genetic modifications may differ:

- History of exposure to chemotherapy or radiation (therapeutic "therapy associated MDS (t-MDS)" or accidental), or both.
- Heavy exposure to hydrocarbons such as in the petroleum industry.
- Exposures to Xylene and Benzene.
- Atomic-bomb survivors may develop MDS; 40 to 60 years after radiation exposure.
- Family history
- Children with DS
- Pre-existing bone marrow disorders (such as acquired aplastic anemia following immunosuppressive treatment and Fanconi anemia).

- Sometimes, patients requiring large numbers of RBCs transfusions can get iron overload on their liver, heart, and endocrine functions. The resulting organ dysfunction from chronic iron overload might be a contributor to MDS.

Genetic changes:

- Loss of chromosome 5 or 7 (may be a consequence of exposure to alkylating agents such as melphalan, busulfan, chlorambucil or procarbazine).
- 11q23 translocation (from exposures to DNA topoisomerase II inhibitors).
- Mutations in the multipotent bone marrow stem cell.
- The loss of DNA methylation control.
- The loss of mitochondrial function over time in older patients.
- 5q- syndrome (the deletion in the long arm of chromosome 5).
- Splicing factors mutations.
- Mutations in the genes encoding for isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2).

II.4.3. MDS Prognosis

Although 30% of patients develop AML, the median survival time of MDS varies from years to months, depending on the type. Older patients have a lower survival rate than younger ones that can receive stem-cell transplantation, increasing it to 50% at 3 years.

Cytogenetic abnormalities can be detected by conventional cytogenetics, a FISH panel for MDS, or virtual karyotype. Nonetheless, the International Prostate Symptom Score (IPSS) is the most commonly used tool in MDS to predict long-term outcome **(Hagemeijer, 1987)**.

Some karyotypic prognostic factors may include:

- **Good prognosis:** normal, -Y, del(5q), del(20q)
- **Intermediate or variable prognosis:** +8, other single or double anomalies
- **Poor prognosis:** complex (>3 chromosomal aberrations); chromosome 7 anomalies

II.4.4. MDS and AML

Most often, AML is related to MDS if some criteria are met, such as the blast count in blood or marrow that should be ≥ 20%.

This case is known as AML with myelodysplasia related changes (MRC).

The present table **(table 08)** points up some cytogenetic abnormalities used to diagnose AML-MRC **(Kerndrup and Kjeldsen, 2003)**.

Table 08: Cytogenetic abnormalities diagnostic of AML-MRC

Type of cytogenetic abnormality	Karyotype
Complex karyotype	3 or more abnormalities
Unbalanced abnormalities	–7/del(7q) del(5q)/t(5q) i(17q)/t(17p) –13/del(13q) del(11q) del(12p)/t(12p) idic(X)(q13)
Balanced abnormalities	t(11;16)(q23.3;p13.3) t(3;21)(q26.2;q22.1) t(1;3)(p36.3;q21.2) t(2;11)(p21;q23.3) t(5;12)(q32;p13.2) t(5;7)(q32;q11.2) t(5;17)(q32;p13.2) t(5;10)(q32;q21) t(3;5)(q25.3;q35.1)

Experimental part

III. 1. Introduction

I was tutored to perform chromosomal analyzes to examine the most recurrent chromosomal abnormalities in patients with different genetic and hematological diseases and who have previously been diagnosed at the “Policlinico Umberto I” University Hospital Center of Rome, then have been sent (as samples) to the laboratory of the University Transfusion Center "Cattedra di Ematologia; U.O.C Immunoematologia e Medicina Trasfusionale".

In this study, we will present the research done in the same laboratory, on seven patients (in whichonly the sex and clinical prognosis were known) including 3 men and 4 women, from March 21st, 2022 to April 7th, 2022, where we attempted to analyze 10 to 20 metaphase cells per patient.

III.2. Materials

III.2.1. Reagents

- Preservative-free sodium heparin (green top tube)
- EDTA
- Growth media (such as MarrowMAX™ Bone Marrow Medium)
- COLCEMID
- Chemical fixative (CARNOYS)
- Hypotonic saline solution (0.9% sodium chloride)
- Trypsin
- Gurr buffer tablets
- Gurr stain Giemsa
- Immersion oil

III.2.2. Equipment

- Bone marrow biopsy/aspiration needle
- Syringe
- Sterile gloves
- Blood collection tubes
- Sterile blood culture bottle
- Incubator
- Centrifuge

- Microscope slides and cover slips
- Pipette
- Test tubes
- Coplin glass staining jars
- Forceps
- 50°C warming oven
- Paper towels
- Light microscope and a computer (ViewSonic Monitor) with CytoVision ®/Genasis software
- Scissors
- Tape

III.3. Protocol for preparing a karyotype

The following scheme summarizes our workflow **(fig. 27)**.

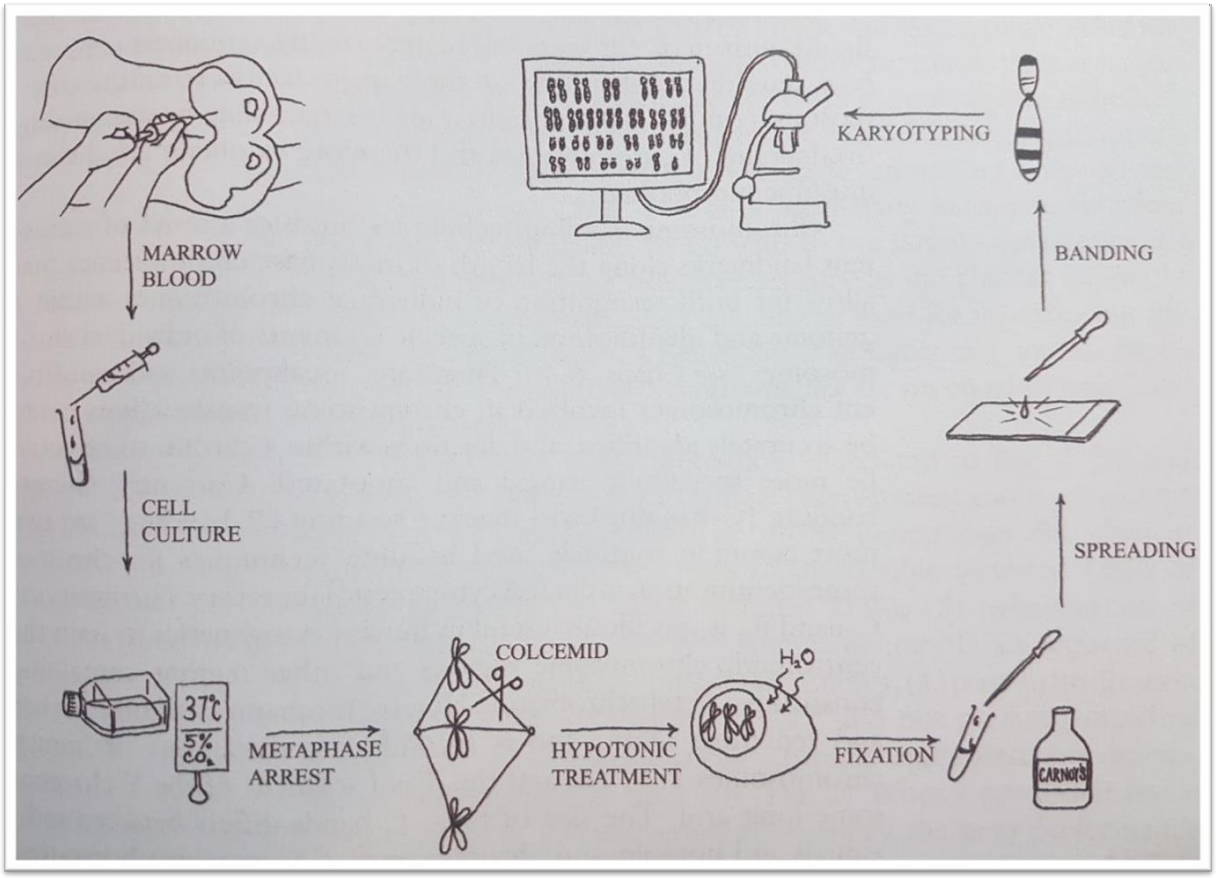

Figure 27: Protocol for the preparation of a karyotype from a leukemic patient **(Wan, 2014)**

III.3.1. Culture process

Bone marrow aspiration biopsies are carried out principally to permit cytological assessment but also for immunophenotypic, cytogenetic, molecular genetics, and other specialized investigations. After choosing the right time and place, it is time to choose the right equipment. Specific biopsy kits are generally provided, and a local anesthesia is performed. The needle is first introduced to the marrow, then, a heparinized syringe is quickly attached to the needle hub to aspirate approximately [1-2]ml marrow.

Next, the marrow is collected in a green top sodium heparin tube, to be cultured in a blood culture bottle using an adequate growth medium (for example, Gibco™ MarrowMAX™ Bone Marrow Medium, provided by ThermoFisher SCIENTIFIC, and mainly developed for the short-term culture of bone marrow and other hematopoietic cells for cytogenetic studies and in vitro diagnostic procedures).

Afterwards, cells are cultured for 24-48 hours at 37°C in a humidified atmosphere (incubator) containing 5% of CO_2.

“For the transportation, we had to avoid freezing or heating over 35°C”.

III.3.2. Harvesting process

Firstly, COLCEMID is added to block the proliferating cells in metaphase, thus allowing an accumulation of cells at metaphase stage.

Second, a centrifuge step at 200 x g is required. Afterward, a removal of the supernatant is done and a resuspension of the pellet followed by an addition of the hypotonic solution to rupture RBCs and swell WBCs are performed, so that another short centrifugation is made.

Carefully, an addition of 5 ml of fresh Carnoy's Fixative (3:1 ratio of methanol:glacial acetic acid) to the cells while vortexing is intended. Then we add 5 ml more of fixative without vortexing for a total of 10 ml.

Finally, a final centrifugation at 200 X g for 5min is necessary (with addition of fixative to each tube and elimination of the supernatant).

III.3.3. Slide preparation and staining process

After gently resuspending the pellet, we can pipette three drops of the cell suspension onto a slide and allow the suspension to roll across the slide.

Accordingly, adding in one large drop of fresh Carnoy's Fixative to the slide ensures the fixture. At that time, the back of the slide must dry, on a paper towel and then, the slide should be placed in the drying oven to be completely dry.

Then we dip the slides in the 0.05% trypsin solution that was freshly prepared, for 5 to 10 minutes.

Preparation of a fresh G banding or Giemsa Staining Solution requires the preparation of 3:1 ratio of Gurr Buffer and Giemsa Stain to stain condensed chromosomes. (Following the procedures provided by ThermoFisher SCIENTIFIC, in the case of using their kit, to immerse the slides in the adequate Jars for adequate times).

Then, while covering the entire slide in the Giemsa staining solution (2% solution of Giemsa in phosphate buffer (pH 6.8)), there is a necessity of letting the slides remain in the staining solution for 5 min. Next, rinsing slides with distilled water is made. At last, we drain, and allow to air dry.

“Coverslips are added to the slides with making sure there are no bubbles in the middle” [8].

III.3.4. Microscopic analysis

Addition of a drop of immersion oil, and analyzing cells with a light microscope under 10X, 100X and 1000X magnifications are thought to be the final step of karyotyping analysis.

The slides are scanned for metaphase spreads. (Usually, 20 cells are analyzed under the microscope) **(fig. 28)**.

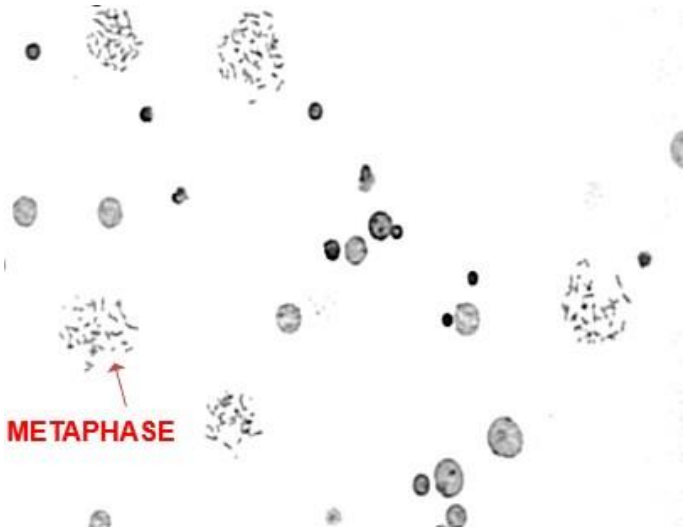

Figure 28: A photomicrograph of metaphase spreading from human bone marrow with low-power (X100) traditional microscopy

After finding a good spread with the minimum number of overlapping chromosomes, a microscopic image is immediately recorded, thereby; using a screen and a specific software **(fig. 29)** provided us with photographed images of arranged chromosomes in a standard presentation format of longest to shortest. Hence, a computer-assisted karyotype is done **(fig. 30)**. (The photomicrographs are in the "results" section).

Figure 29: Light Microscope connected to a computer with specific software

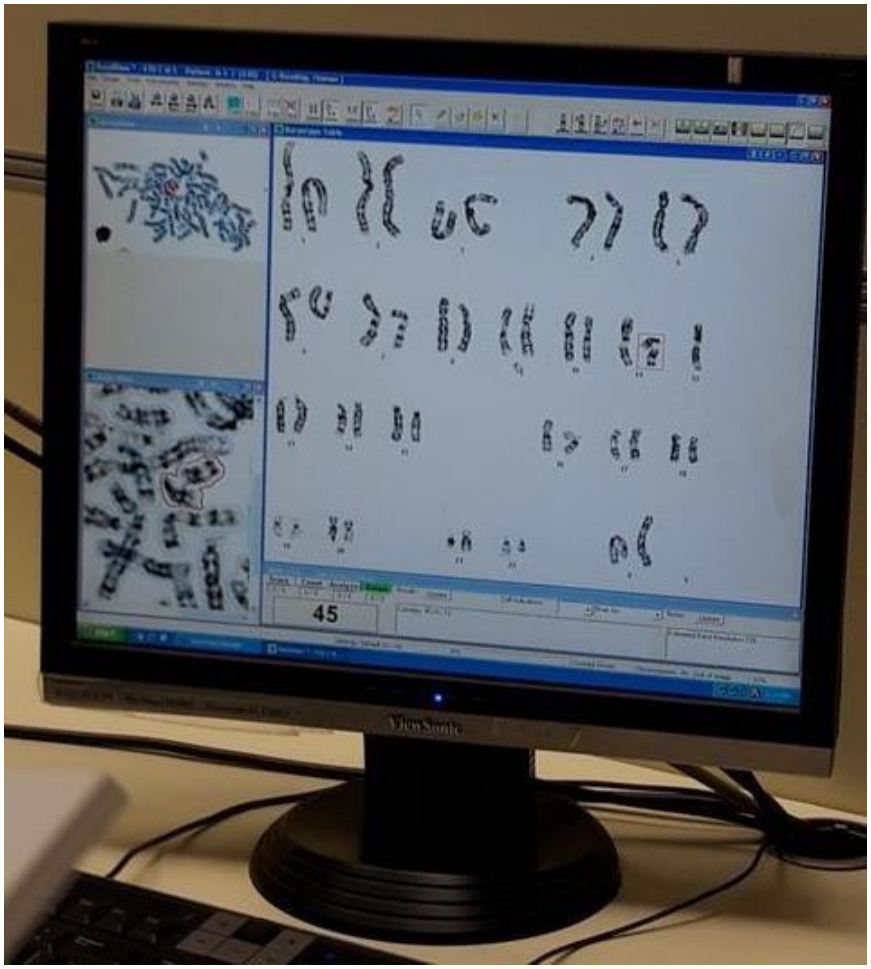

Figure 30: A computer-assisted female Karyotype **45, XX**

III.4. Reviewing

Additional human efforts are usually carried out in detecting chromosomal abnormalities, because, the computer can commit mistakes especially in the presence of overlapping or very damaged chromosomes. Besides, FISH analysis should be incorporated too, to detect specific target arrangements as well as chromosomal number changes.

I tried to distinguish chromosomes and locate CAs manually by cutting all chromosomes separately from the metaphase spread (printed papers), and sticking them on a separate blank paper to visualize the karyograms properly after arranging them in pairs from chromosome 1 to 22 and sex chromosomes in last, using the normal karyotype as a reference **(fig. 31.A.B.C.)**.

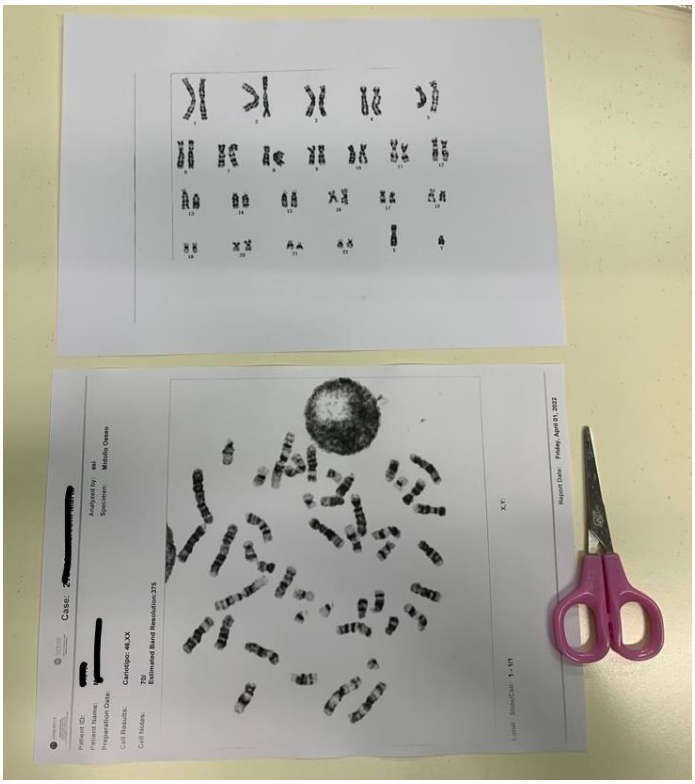

Figure 31. A: 1st step to obtain a karyotype performed manually: a metaphase spread and a normal karyotype

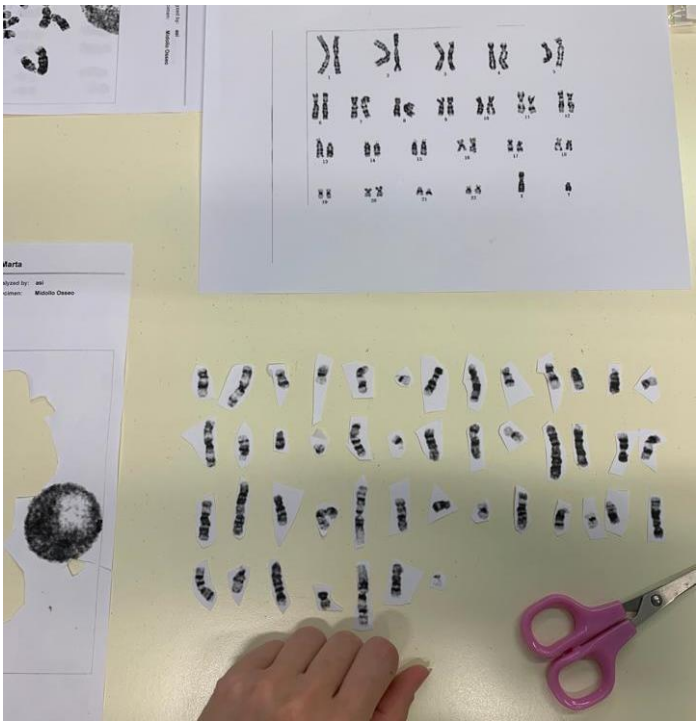

Figure 31. B: 2nd step to obtain a karyotype performed manually: separating chromosomes

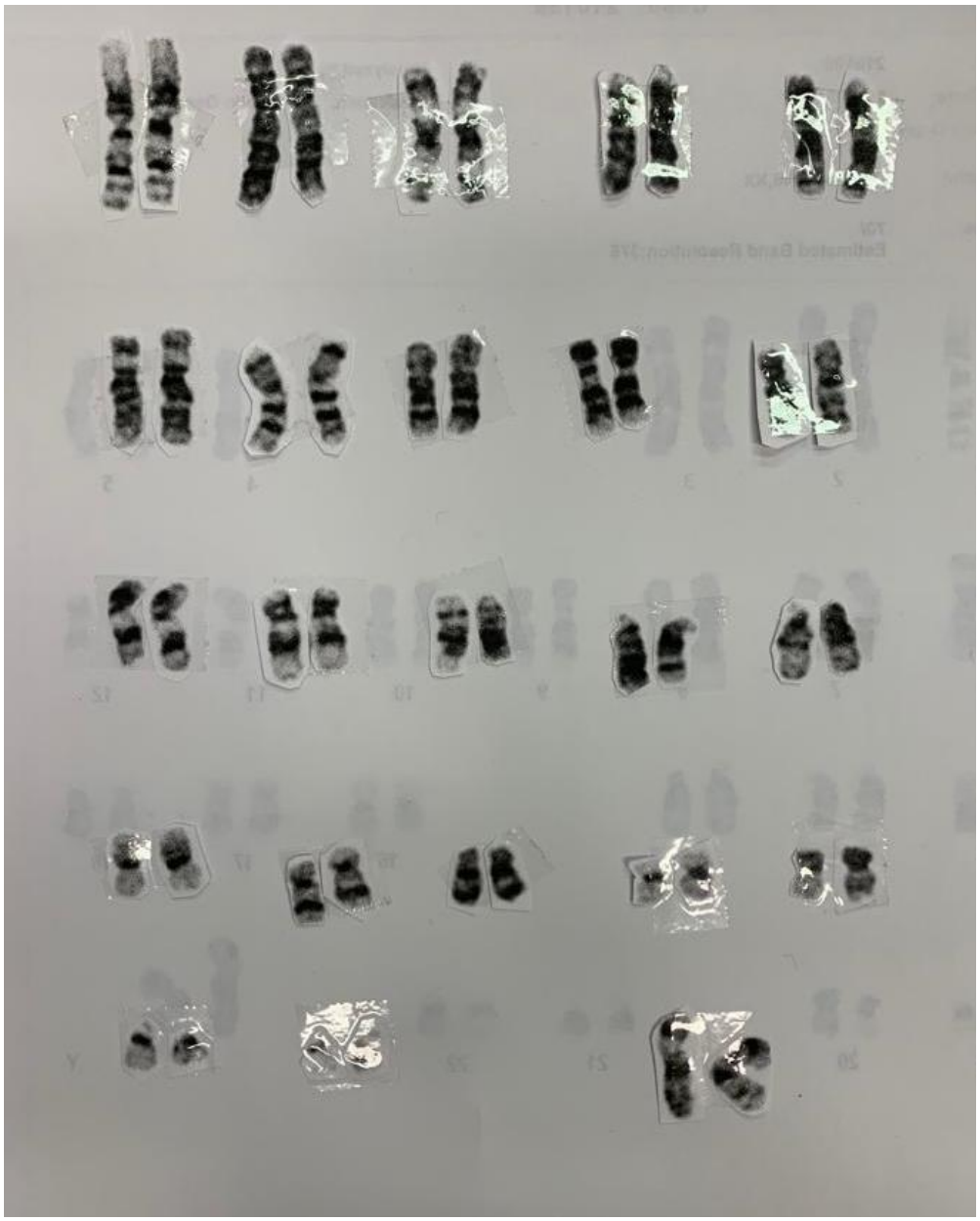

Figure 31. C: 3rd step to obtain a karyotype performed manually: arranging chromosomes in their standard presentation format

Results and Discussion

IV.1. Results

Karyotypes were designated according to the latest version of ISCN. And chromosomal abnormalities were defined as clonal if two or more metaphase cells had identical structural abnormalities or extra chromosomes, or if three or more metaphase cells had identical missing chromosomes.

IV.1.1. Normal Karyotype

There is always a normal karyotype representing a healthy person's chromosomes isolated from an individual cell to compare to.

Here is an example of the normal karyotype that we worked on, and which was obtained from WBCs of a healthy male **(fig. 32)**.

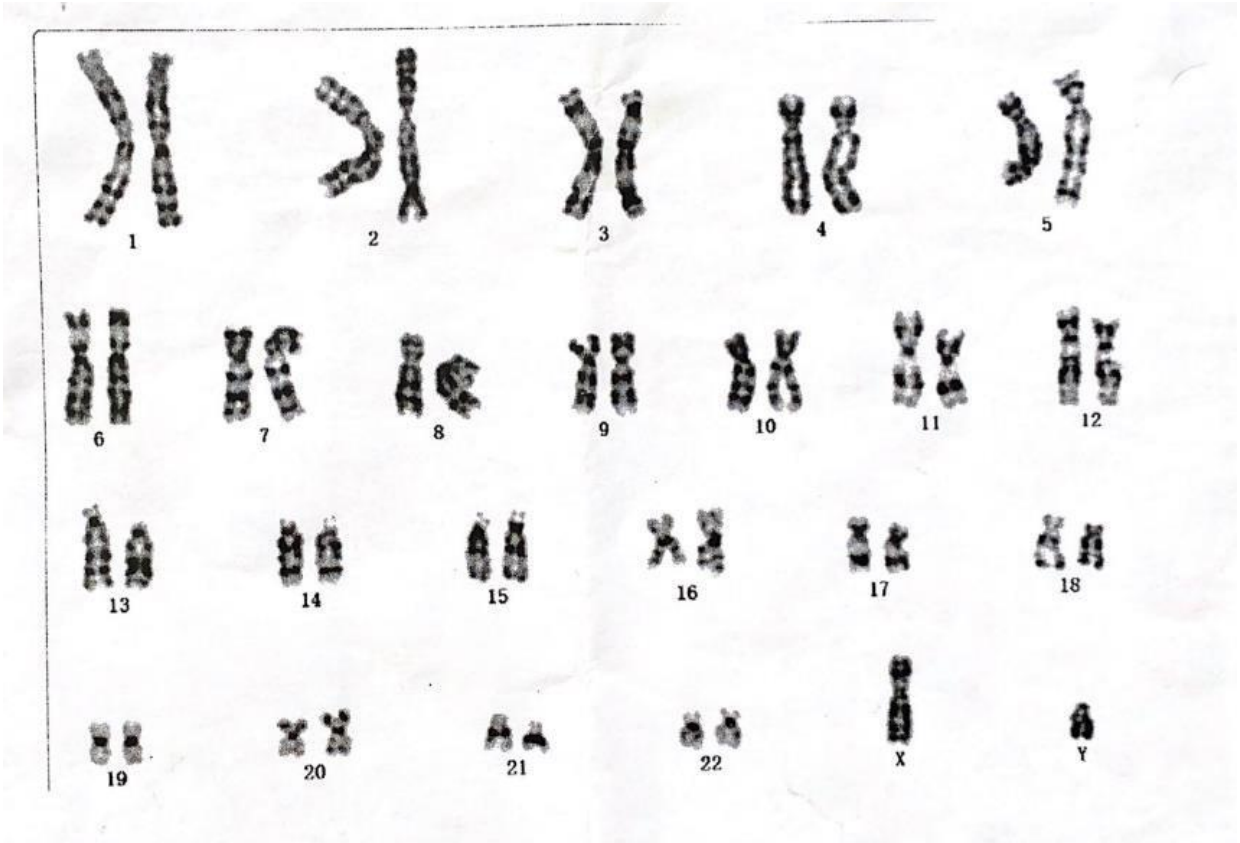

Figure 32: G-banded karyogram of a normal male cell showing the band characteristics of each pair of homologues, as well as of the sex chromosomes (X and Y)

Heterochromatic regions, which are staining more darkly in G-banding tend to be rich with adenine and thymine (AT-rich) DNA and relatively gene-poor. In contrast, less condensed chromatin (Euchromatin) which tends to be rich with guanine and cytosine (GC-rich) and more transcriptionally active are incorporating less Giemsa stain, so they are the regions that appear as light bands.

IV.1.2. Abnormal Karyotypes

Analyzing the results of 7 patients that were obtained after G-banding procedure:

Patient 01: Case N° 210198

The first analyzed specimen had a normal karyotype in some clones:

46, XX; **(fig. 33)**.

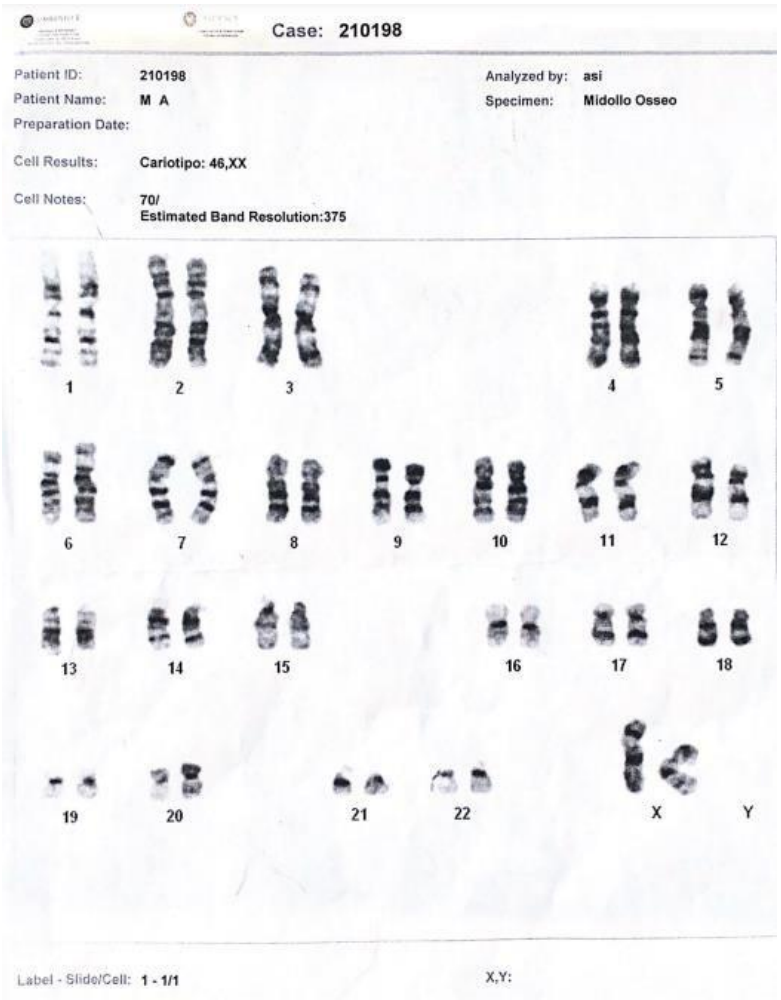

Figure 33: A photomicrograph of a human female karyotype **46, XX** showing no CAs

In the other hand, another clone of the same patient, showed different karyotype **(fig. 34)**:

52, XXX, +6, +14, +21, +21, +21

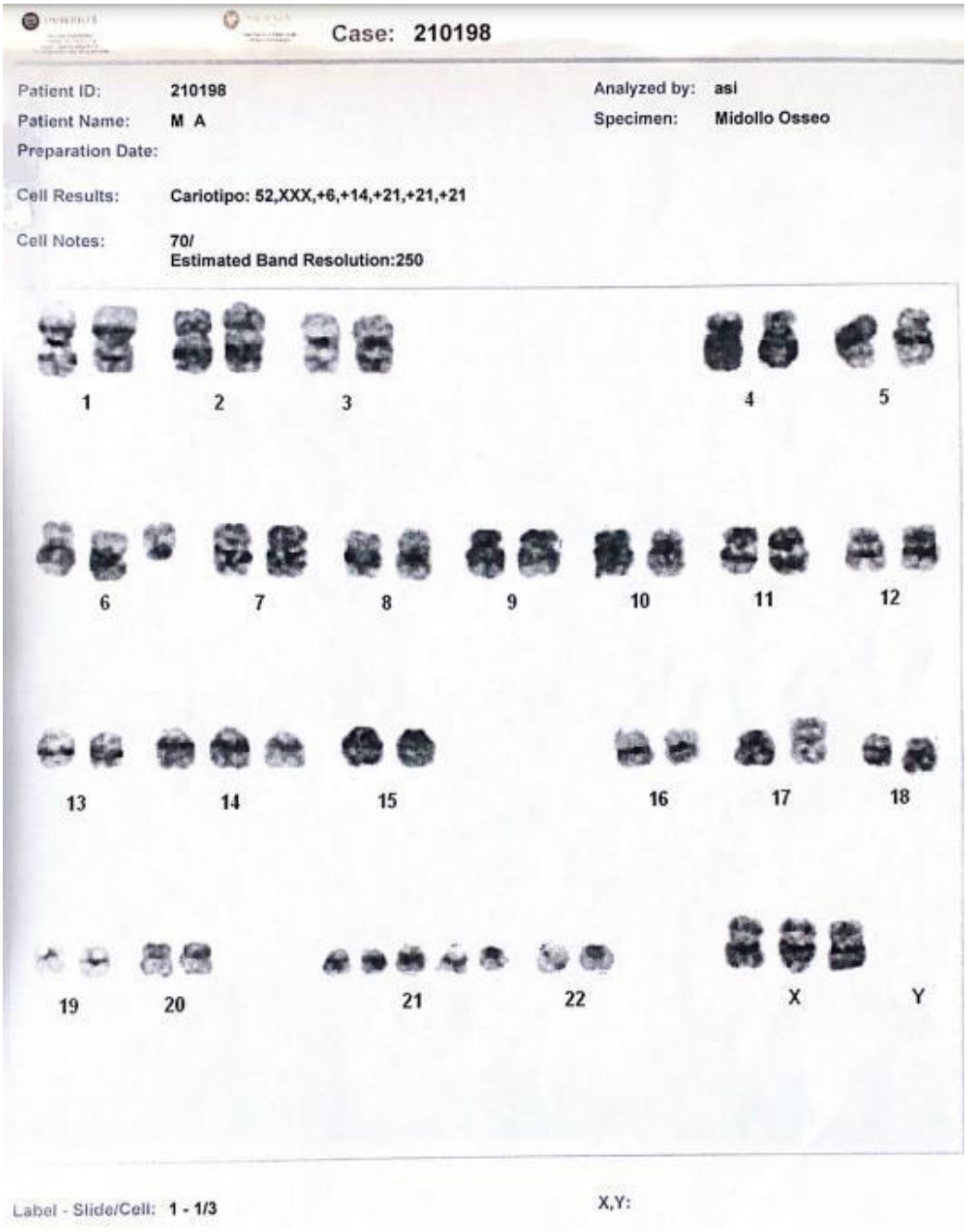

Figure 34: An abnormal karyotype of a human female showing CAs and high hyperdiploidy

We can estimate the high number of extra chromosomes, for a total number of 52 chromosomes with 3 sex chromosomes. These features are characteristic of ALL disease.

More precisely, this patient has been diagnosed with **B-Cell ALL**.

Patient 02: Case N° 210422

The following metaphase spread **(fig. 35)** was collected from cells of a human male:

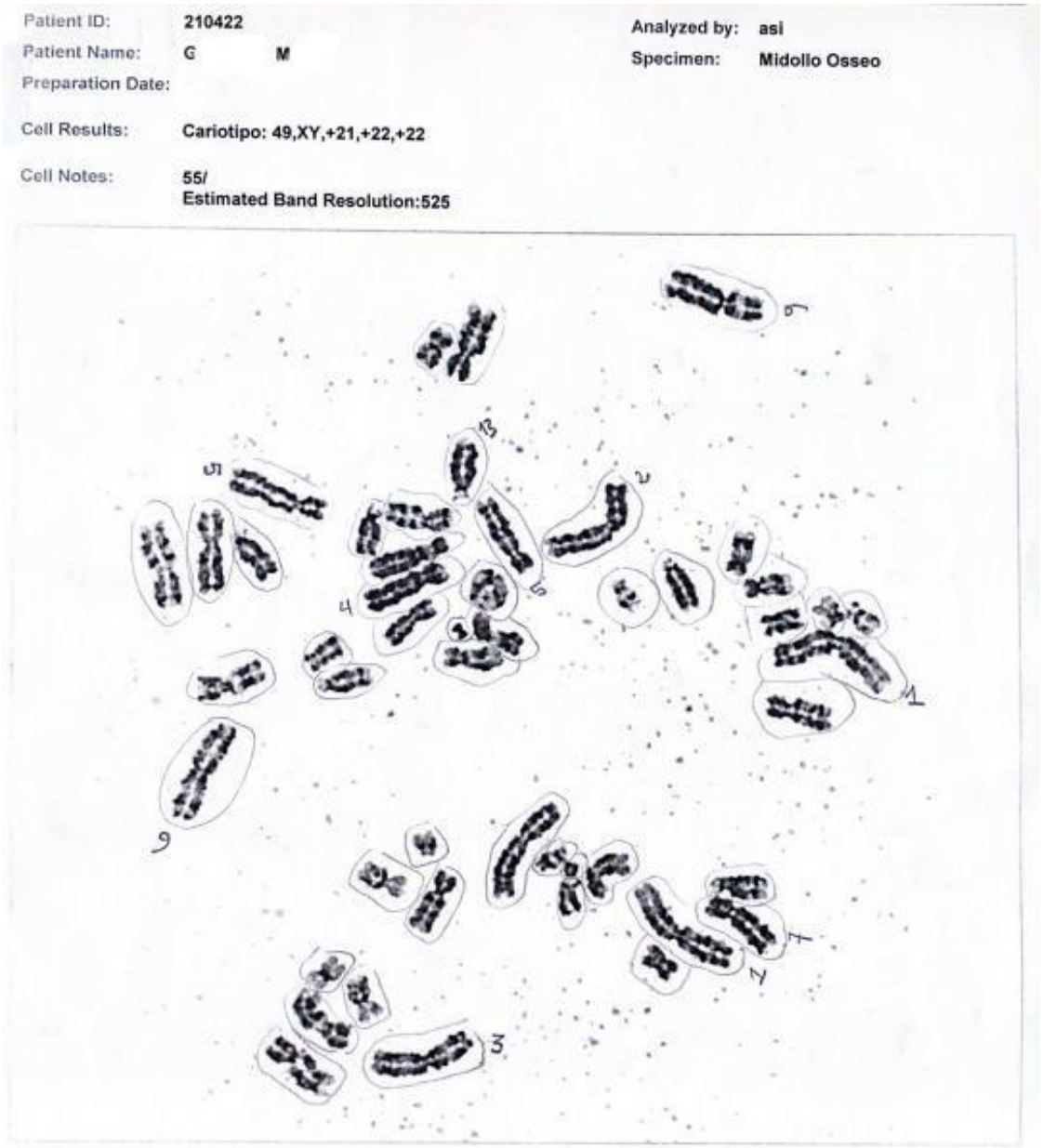

Figure 35: A photomicrograph of a metaphase spread of a human male

49, XY, +21, +22, +22

Later, after obtaining the automatically arranged picture of the chromosomes, we noticed that there are 3 gains here: 21, 22, 22. **(fig. 36)**

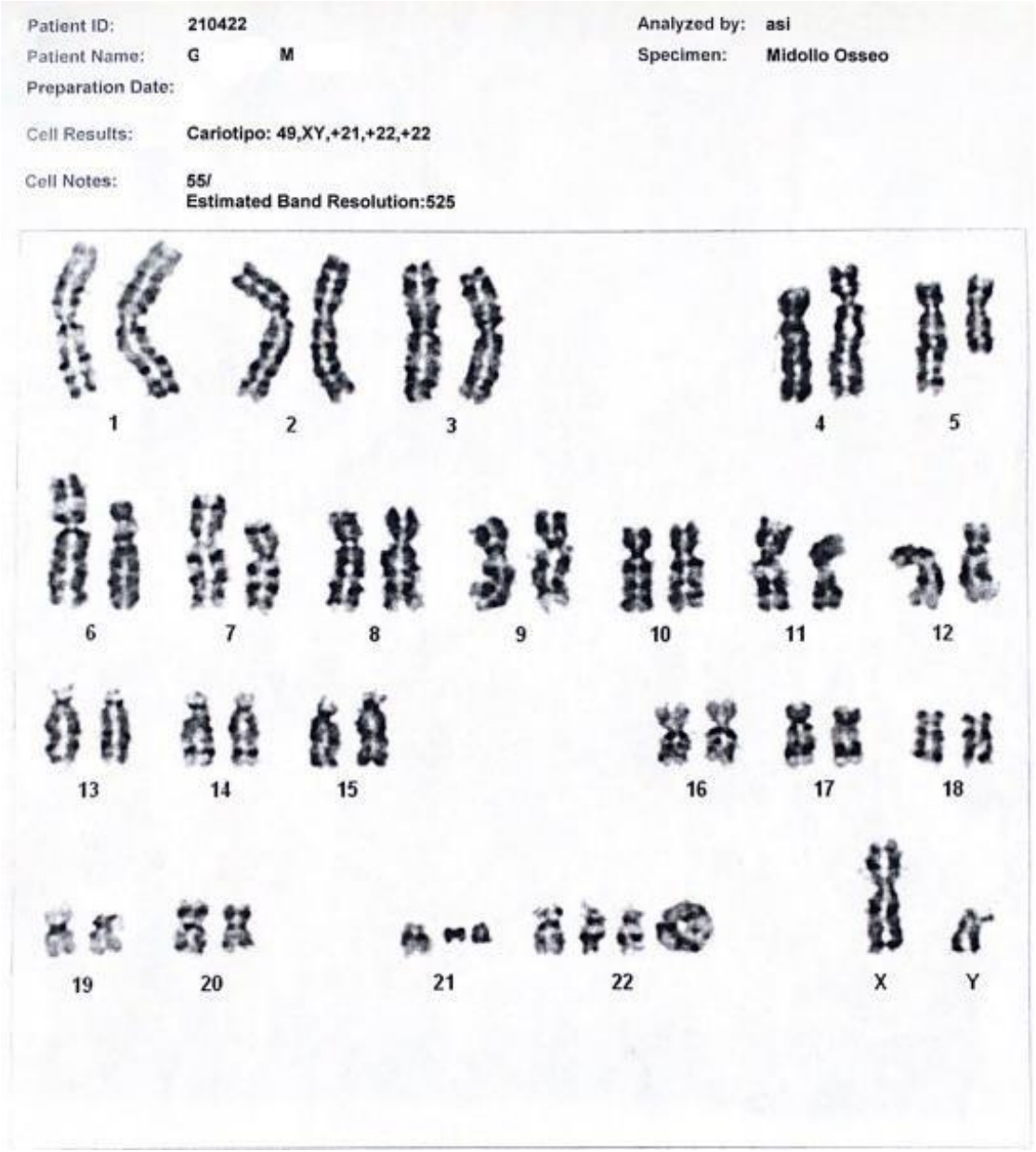

Figure 36: An abnormal karyotype of a human male

Chromosome analysis of this case showed a complex karyotype with multiple numerical and structural rearrangements including translocations (that were confirmed by FISH analysis).

We can observe the presence of a ring chromosome as well.

This karyotype is associated with **DLBCL disease** and the implicated oncogenes are determined by whole-genome sequencing (WGS) and whole-exome sequencing (WES) as it has tremendous genetic heterogeneity.

Patient 03: Case N° 210389

The following figure **(fig. 37)** reveals the chromosomes that I cut from the metaphase spread of the 3rd patient's cells, in order to arrange them and perform a karyotype.

Figure 37: A set of an individual's chromosomes

By counting the chromosomes, we can directly determine that there are 46 chromosomes with XX chromosomes, so this specimen is a female.

Yet, analyzing the G-banded chromosomes helped us to define the chromosomal structural changes **(fig. 38)**. By so, we discovered the presence of a small chromosome in the 22 chromosome pair, that resulted from the reciprocal translocation between chromosome 9 and 22 "**t(9;22)(q34;q11)**".

The truncated chromosome is the one known as the **Philadelphia chromosome (Ph)** and is a hallmark of **CML** malignancy.

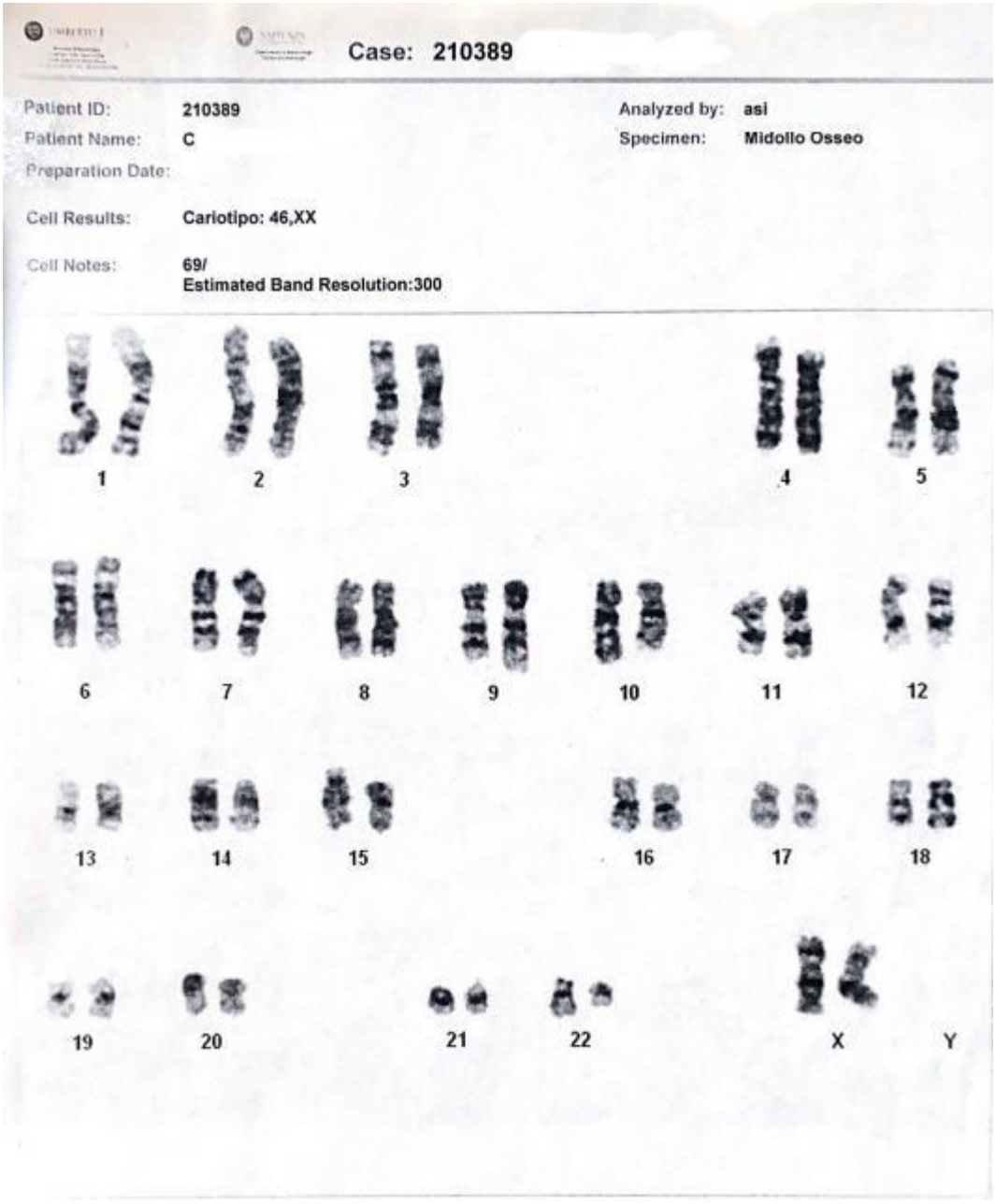

Figure 38: A karyotype of a human female **46, XX** showing Ph Chromosome

This karyogram had to be confirmed with other analysis such as FISH technique to approve the presence of the fusion gene which was created by juxtaposing the ABL1 gene on chromosome 9 (region q34) to a part of the BCR (breakpoint cluster region) gene on chromosome 22 (region q11).

Patient 04: Case N° 220066

Moving to another case, which is a human female and she has been diagnosed with **MM** at first, but the disease has developed to **MDS** and then to **AML**. The figure below shows her complex karyotype **(fig. 39)**.

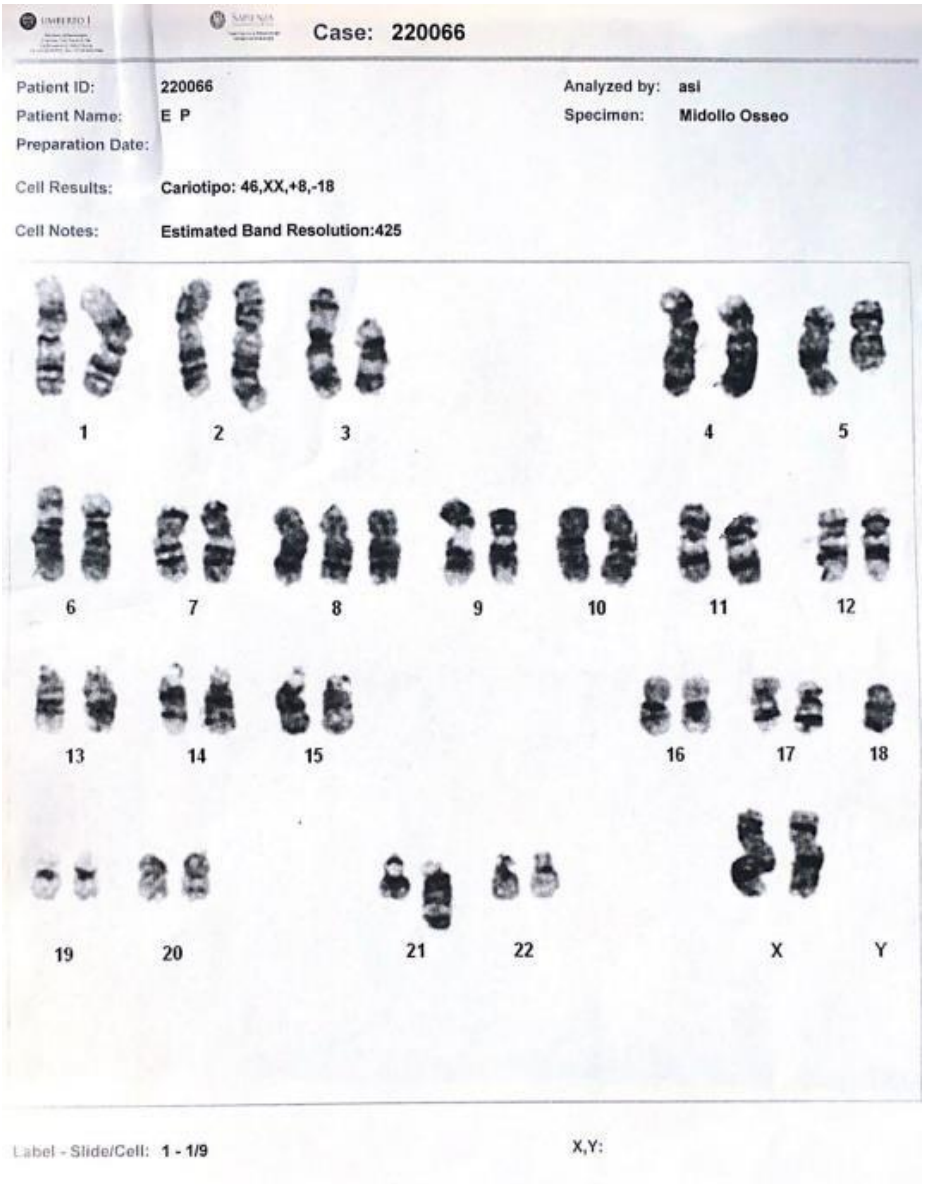

Figure 39: A female karyotype **46, XX** with structural and numerical CAs

As we can see, numerous translocations, deletions, insertions and inversions exist. We noticed also chromosome gain and loss. We can summarize these CAs as follows:

Ins(2;7)(q33;q22), del(3)(p21), del(5)(q13 ;q31), t(6;?)(p21;?), +8, -18, der(21)add(21)(q21.3)del(21)(q21.3), inv(X)(q13 ;q28)

The « add » abbreviations refers to unidentified additional material.

Furthermore, trisomy 8 is the most common karyotypic finding in AML and MDS disorders, occurring in approximately 5% and 10% of the cytogenetically abnormal cases, respectively [9].

Patient 05: Case N° 220023

The 5th patient was a male. Here are his results **(fig. 40)**:

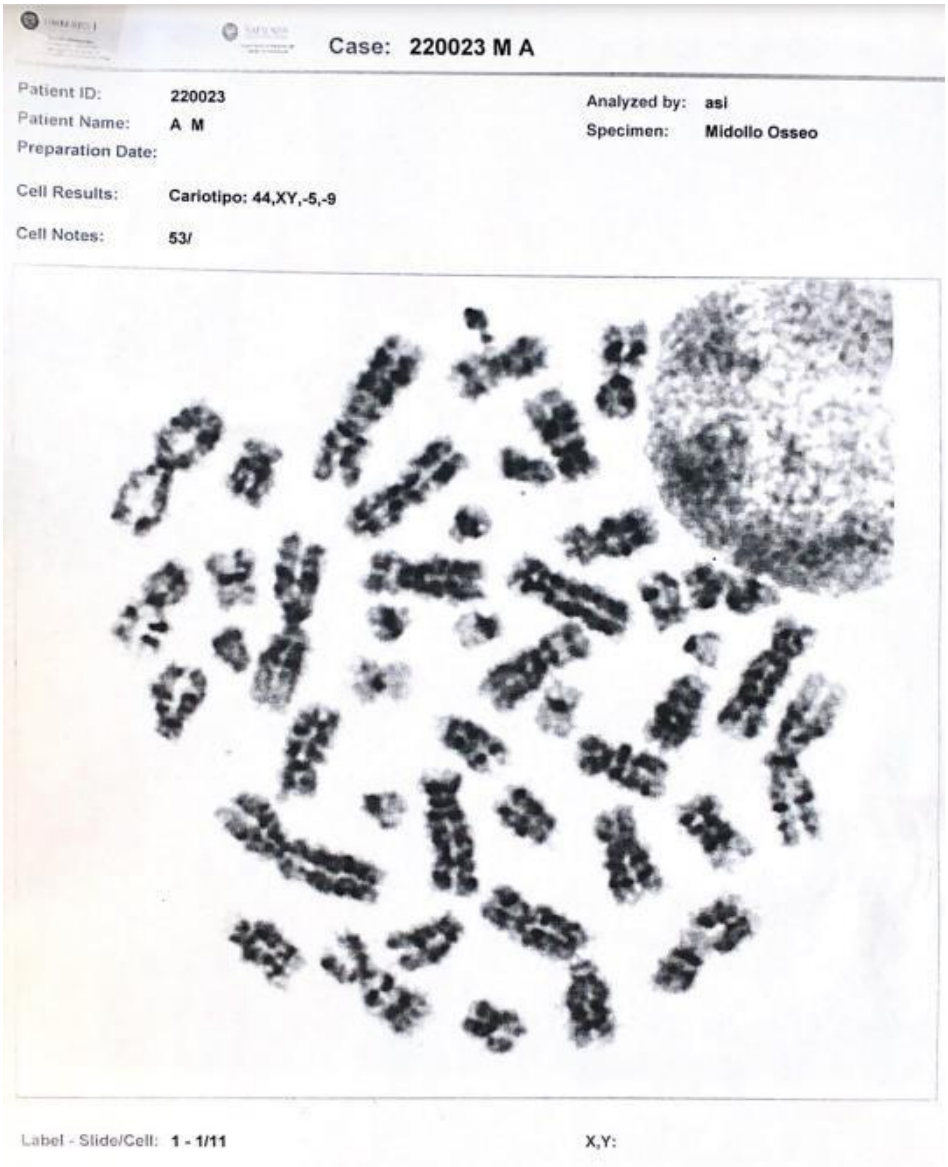

Figure 40: A photomicrograph of a metaphase spread of a human male **44, XY, -5, -9**

As we can, the chromosomes are well stained with G dyes, to be counted and analyzed based on their bands. A karyotype is realized, showing loss in chromosomes 5 and 9 **(fig. 41)**.

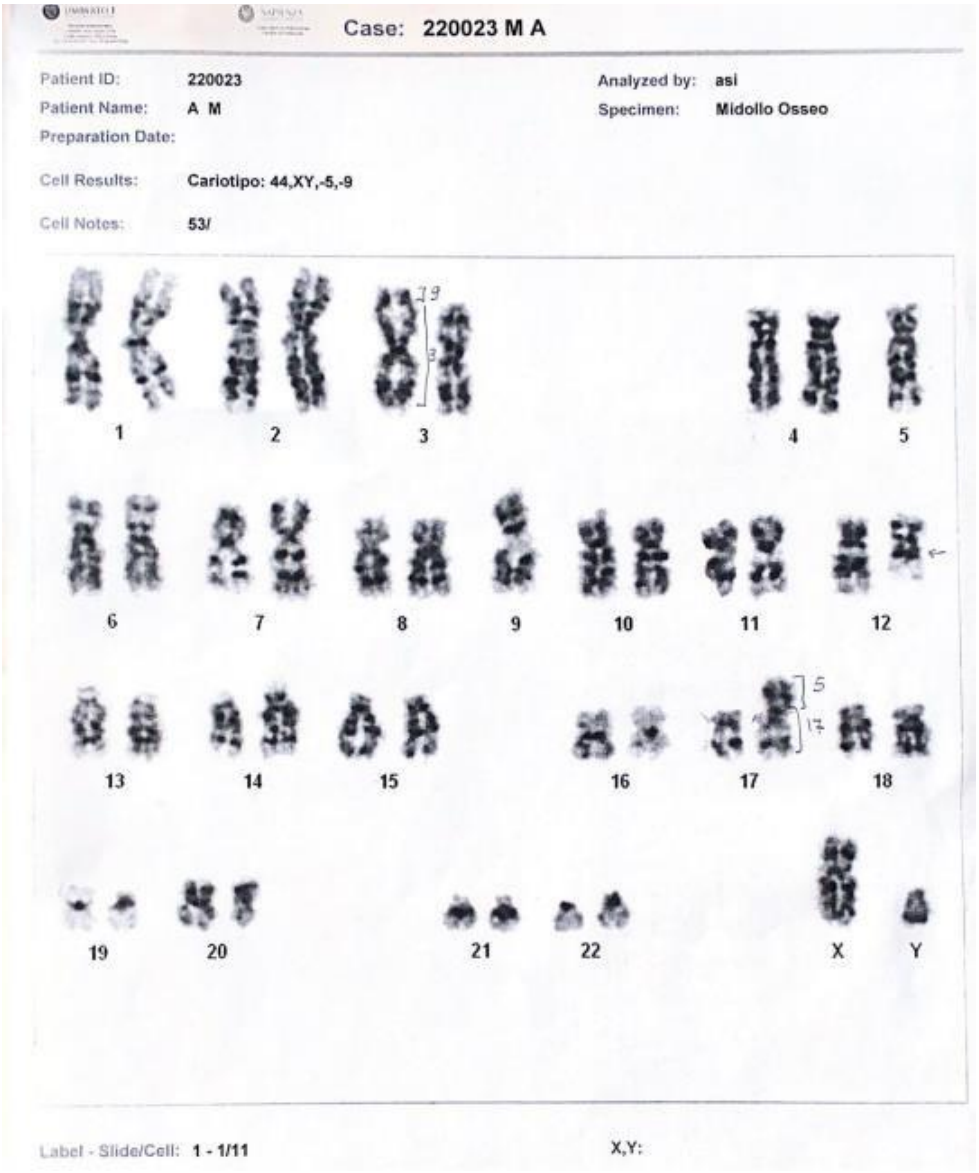

Figure 41: A male karyotype **44, XY, -5, -9**

The cytogenic analysis of samples from patient N°5 demonstrated that:

It is a male with several structural and numerical changes in his karyotype. He has been diagnosed with "**MDS with complex karyotype"** (bad prognosis), since he had more than 3 abnormalities:

44, XY, der(3)t(3;9)(?;?), -5, -9, del(12)(q13q14), der(17)t(5 ;17)(?;p12)[5] / 46, XY

Patient 06: Case N°210030

The next chromosomes were again extracted from a male's cell **(fig. 42)**.

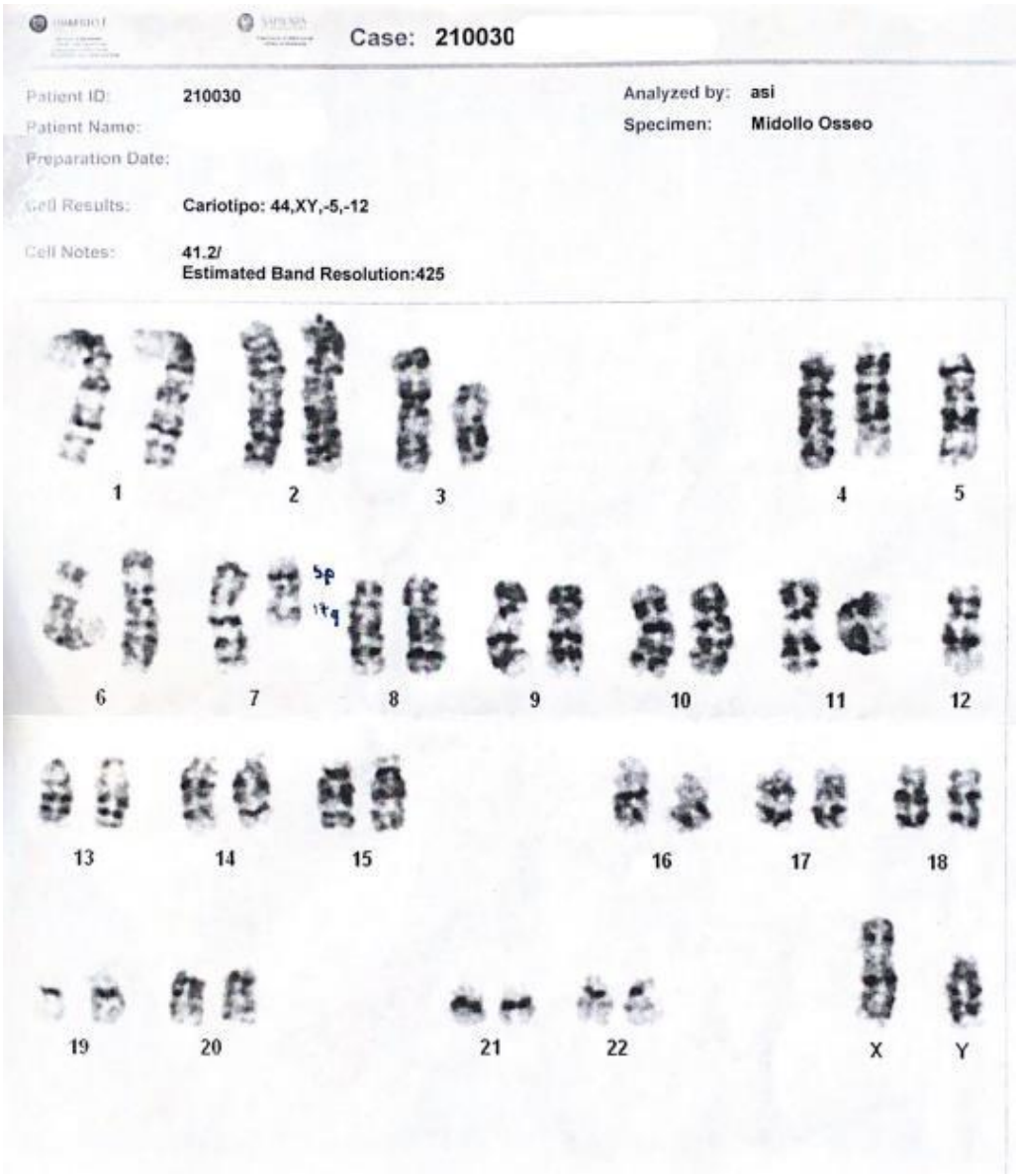

Figure 42: A karyotype of human male **44, XY, -5, -12**

The present karyotype indicates the existence of many genetic changes including insertions, deletions and translocations, such as the chromosome in pair 7, where we can see that there is an **insertion of 5p and 17q**, while one of chromosomes 5 pair is missing, in addition to a loss of chromosome 12.

This specimen has **MDS** (based on the structural ana numerical changes in his chromosomes).

Patient 07: Case N° 111357

The last patient was a female; **47, XX (fig. 43)**, suspected to have **MDS**.

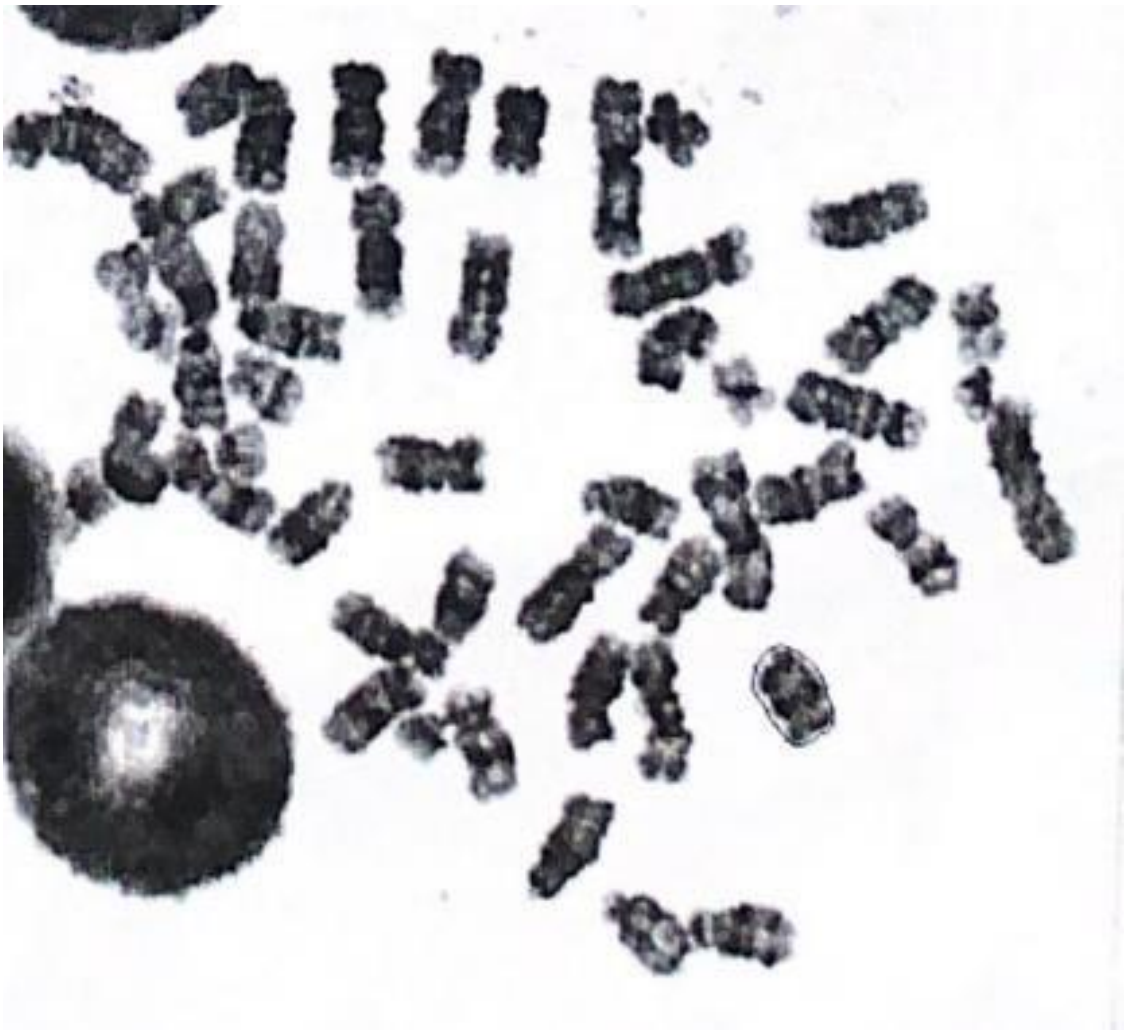

Figure 43: A photomicrograph of metaphase spread karyotyped: **47, XX, +8, +8, -20**

After analyzing this metaphase and performing the karyotype, the cytogenitist was able to detect chromosomal rearrangements and among the most important are:

2 gains in chromosome 8 and a loss in chromosome 20 (fig. 44).

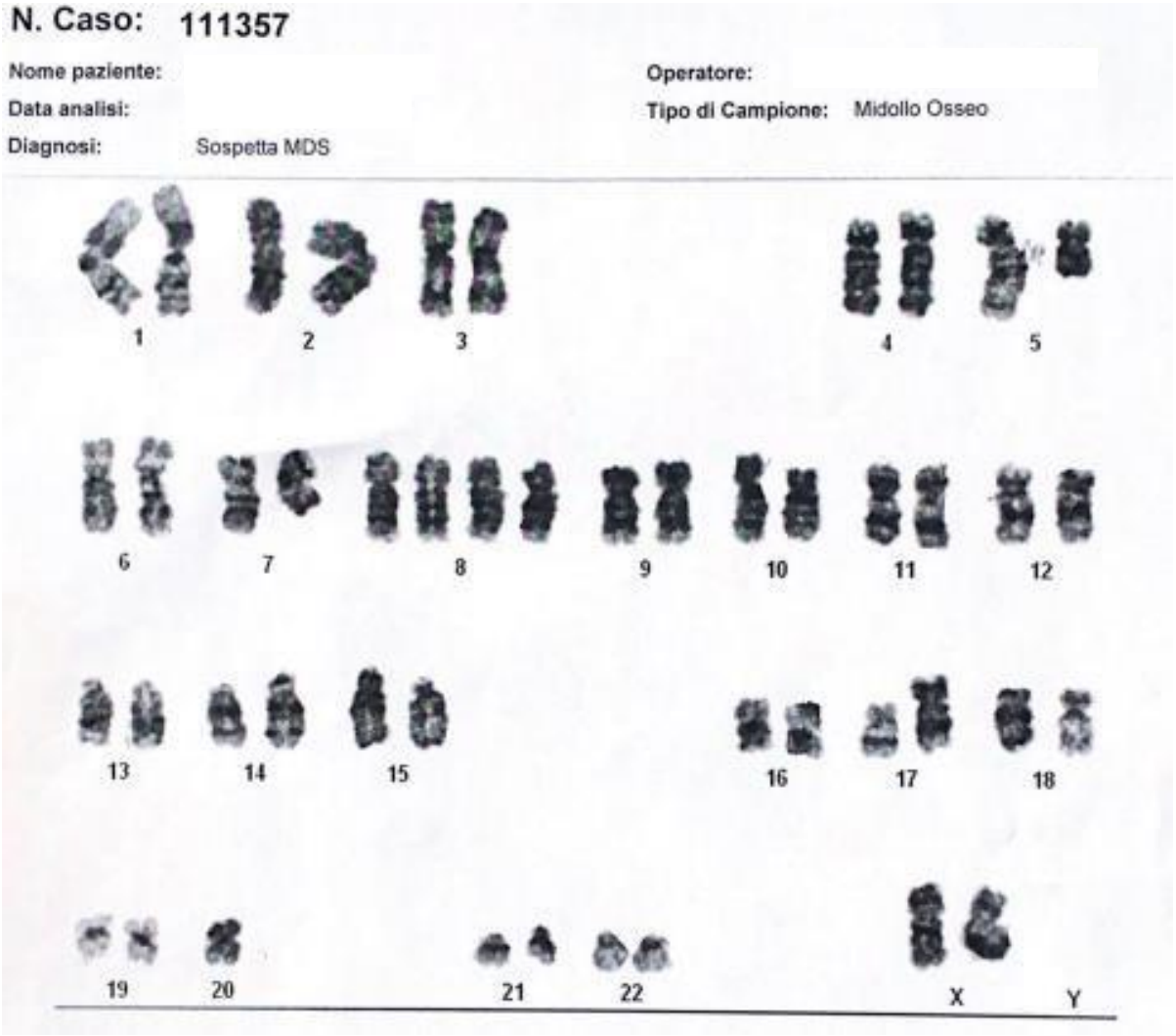

Figure 44: A female karyogram **47, XX, +8, +8, -20**

It demonstrates the most common karyotypic lesions in MDS, involving chromosomes 8 (gain), 5 and 7 deletions.

IV.2. Discussion

Cytogenetic analysis, the study of chromosomes by G bands, is used for karyotype analysis of abnormal cells, then it can be combined with FISH analysis to detect any genetic change.

Since we could determine the presence of malignant clones, this approach allowed us to agree on the fact that cytogenetic analyses can provide valuable and extremely suitable information of the analyzed genomes. In this way, the physician who collected samples from 7 patients and sent them to be cytogenetically analyzed can affirm the presence or the absence of the malignancy.

First, the selection of our samples for cytogenetic testing had a specific purpose which is to detect CAs in hematological diseases. Usually, bone marrow is the specimen of choice for leukemias and lymphomas (whether by aspiration of the fluid or biopsy of the cells). Because the length of in vitro culture depends on cell type, so, we used bone marrow samples as they are unstimulated and they contain spontaneously proliferating cells. They are harvested at 24 hours and if volume is sufficient, a 48-hour culture is also initiated followed by G-banding technique that produces specific alternating bands along each chromosome. [10]

Next, cell analyses of healthy people often show that they have normal karyotype with the correct number and structure of the complete set of chromosomes, 22 pairs (autosomes) and a pair of allosomes (sex chromosomes: X and Y), or they may be carriers of very small genetic changes. However, some karyotypes of unhealthy people may mimic the normal karyotype in some of their cells, and that was the case with the first patient that had at first place a normal karyotype with 46, XX, but in which, there was a masked hyperdiploid. In the same time, recent studies have demonstrated that a doubled clone can sometimes be mistaken as a typical hyperdiploid cell.

Based on both our findings and the reports available in the same context, we approve that in hematological disorders, particularly in acute leukemias, abnormal and normal karyotypes may be present in the same sample. That is why, we thought of the necessity of applying and testing samples with more sophisticated molecular cytogenetic techniques which can lead to getting the right interpretation of results, thus, right diagnosis of disorders.

Moreover, Attention is highly recommended in this type of analysis. Any small change in the procedures such as specimen transport to the cytogenetics laboratory in the inappropriate medium, microbial overgrowth, and technical errors involving cell harvest, slide preparation, or staining could result in assay failure. From our work, we assume that few additional minutes of COLCEMID treatment (that inhibits mitosis at metaphase by inhibiting spindle formation) can alter the obtained karyogram, not in the chromosomes' number or structure, but, in their size. They will appear shorter and thicker than they actually are, this was observed in the karyogram of the same patient (N°1). As a consequence, the crucial factor for the G banding is the wise use of time.

Additionally, cytogenetic analysis can sometimes overcome a misdiagnosis. Meaning that, an abnormality could be overlooked or incorrectly interpreted. For this reason, while inconclusive results are determined, checking out with molecular cytogenetics' tools is recommended to help or verify chromosome rearrangements. Nevertheless, and most importantly, the morphologic interpretation and correlation of results on all cases must be carried out by a qualified cytogeneticist and revised by a board-certified scientist (he may be the laboratory director).

This work was designed to investigate the CAs implicated in several hematologic/genetic disorders and the obtained experimental data were similar to a great number of research that have been performed to detect and locate recurrent balanced and unbalanced chromosomal changes leading to different hematological and genetic disorders:

Among the identified chromosomal alterations in DLBCL: gains (19p, 21, Y, etc.) and many translocations such as [t(7;15)(q22;q22)], these outcomes were similar to the findings reported in a scientific article about Chromosome abnormalities in DLBCL **(Zhao et al., 2013)**.

Concerning CML, the presence of Ph chromosome is its main hallmark, where it is found in more than 90% of the diagnosed cases **(Hagemeijer, 1987)**.

Thereafter, chromosomal abnormalities were detected in 4 patients diagnosed with MDS pathologies, including the deletions of chromosome 5q and 7q, trisomy 8, and complex

karyotypes. These marks matched the results provided in “Genetic abnormalities and pathophysiology of MDS” research, **(Hosono, 2019)**

Gathering all the results above, and because those chromosomal abnormalities can have many different effects that result in several malignancies, depending on the specific abnormality, it is important to call attention to the cytogenetic testing and its importance in confirming the findings of complete blood count (CBC) or other tests used to spot diseases like lymphomas and myelomas. In this way, the cytogeneticist ascertains the pathology diagnosed by a physician and indicate the chromosomal rearrangements involved in it. This will offer a clearer idea of the biology of the malignancy and its prognosis, which helps the doctor in finding the specific treatment and predicting the response to it.

Last but not least, in the genome sequencing era, molecular cytogenetics and microarrays are the best way of determining any hematological disorder, so that personalized therapy can be realized to treat those affected.

General conclusion and prospects

Conclusion and prospects

Even though karyotype analyses can be more cost-effective when analyses must be performed on a large number of cells from a heterogeneous population, but they occupy an important position in genomic analyses and can be combined with higher resolution molecular methods that focus more on submicroscopic level changes in detecting certain disorders.

Recent studies reveal that complex karyotypes are associated with unfavorable prognosis, and thus are considered independent prognostic markers regardless of the disease type. In the same time, blood disorders had shown critical and recurrent chromosomal changes that became hallmarks of certain hematological disorders.

Risk Factors and causes of leukemias and lymphomas are not yet fully known, yet, exposure to radiation or certain chemicals, chemotherapy in the past, etc., can contribute to these types of cancer. Sometimes, even certain blood disorders such MDS can develop into other malignancies [11]. Although, smoking is an important contributing factor in many types of cancer, such as lung cancer and acute leukemia as it weakens the immune system. However, there is no proven link between alcohol consumption and an increased risk of any type of leukemia, including AML. Still, the presence of a risk factor does not mean the certainty of falling ill, but a greater probability than the absence of such a factor.

The highlighted hematological diseases are diseases that keep the body from making normal, healthy cells. Eventually, a person will start to lack RBCs that carry oxygen, platelets that prevent easy bleeding, and WBCs that protect the body from illness. The result can be deadly. In 2019, a total of 6,348 deaths from leukemia were registered in Italy. Deaths from leukemia seemed to be more common among males than among female [12].

Moreover, if we do not detect any cytogenetic alteration, we cannot conclude that there is an absence of a particular disease or that the prognosis is better than if a genetic abnormality was observed. For example, in ALL there are many different genetic rearrangements that are each associated with a different outcome.

Our investigations aimed to focus on the applied filed of Molecular and cellular biology. In our point of view, further technological advance should be made to overcome the limitations

of diverse existing techniques. Consequently, gathering both known and newly incorporated techniques in the fields of bioinformatics, genomics, conventional and molecular cytogenetics would help to diagnose instantly the malignant disorders that resulted from genetic rearrangements.

Finally, since detecting CAs can facilitate the screening and diagnosis of the suspected disorder, so combination of conventional and molecular cytogenetics is really an informative way that enables physicians to confirm the diagnosis and assess the prognosis, thereby, choose the specific treatment and predict the response to it. By so, development into other malignancies could be restrained. Inevitably, a close relationship between the pathologist and the cytogeneticist is essential if maximum useful information is to be produced from the cytogenetics studies, especially of hematological diseases. Even though in many cases there is no cure for CAs. However genetic counseling and physical therapy may be recommended. Accordingly, targeted therapy can take place, and introducing personalized medicine would improve the survival rate.

From our perspective, a true understanding of all that is happening at the molecular level, the ongoing exploration of the fundamental epigenetic events involved in genetic aberrations, and the pursuit of investigative clinical studies are helping to develop new strategies for the diagnosis, prevention and treatment of human diseases. Due to the fact that in recent times, they are already using epigenetically targeted therapies in clinical trials, which, at least for now, are showing promising results in hematological malignancies.

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