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Immunophenotyping and Minimal Residual Disease in Palestinian children under 15 with Acute Lymphoblastic Leukemia: A Retrospective Study from Beit Jala Governmental Hospital

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This thesis was submitted in partial fulfillment of the requirements for Master's degree in Health Informatics

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Thesis Approval

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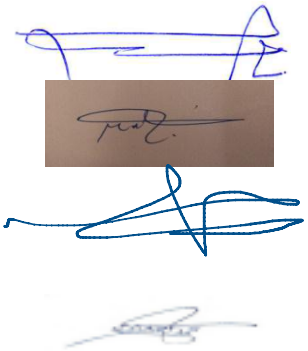
1. Dr. Thaer Abdelghani / Supervisor	
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Declaration

This thesis was submitted in partial fulfillment of the requirement for Master's degree in Health Informatics.

I declare that the content of this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

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Dedication

To the first teacher of all people, Prophet MOHAMMED (peace is upon him), my dear father and mother, my beloved sweet family, my husband and child who supported and encouraged me at all stages, my friends, colleagues and all who helped me in completing this study.

Acknowledgment

To my father; the one who spent the years of his age with giving, tiredness, love, affection and tenderness for our sake.

To my mother; the wonderful and patient person, who was supportive with her words, tender in her affection, big in her love.

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Abstract

Background

Leukemia is an abnormality of many blood cells because of a defect in bone marrow. It influences mostly elderly people aged 55 years and above but it is also a known cancer in children less than 15 years. The main goal of this research is to test the Minimal Residual Disease (MRD) at the day 33 after treatment. MRD is a test that detects the existence of leukemic cells (B-cells, T-cells, and precursor B-cells) remain in a blood of patient and classified as a main reason of relapse in leukemia, and could be a significant effect in specifying the protocol of treatment.

Goal

This study defines the dominant immunoglobulin markers among targeted patients and examined white blood cells count, hemoglobin, and platelets count. The method used in this research is to detect minimal residual disease was a flow cytometry with eight colors. This quantitative descriptive design approach retrospective study focused on a common cancer for pediatric which was childhood acute lymphoblastic leukemia.

The main goal of this research is to test the Minimal Residual Disease (MRD) after treatment at the day 33. The MRD is a test that detects the existence of leukemic cells (B-cells, T-cells, and precursor B-cells) remain in a blood of patient and classified as a main reason of relapse in leukemia

Study population

It targeted 84 patients; 44 males and 40 females. Their data were collected from medical records stored in Beit Jala hospital. Samples were taken in Beit Jala hospital and sent to King Husein Cancer Center in Jordan, and King Hussein Cancer Center sent back reports to our hospital.

Results

This study results showed that MRD tested positive in 18 patients with 21.4%from the total population. MRD was positive in 11.8% patients who had B-cells, 6% patients in precursor B-cells, and 3.6% patients with T-cells.

The B-cell appeared in 54.8%, precursor B-cell constituted 27.4%, and T-cell showed in 16.6%, and one patient with down syndrome diagnosed as B-cell with 1.2%.It is also shown that some markers such as CD10, CD34, CD20 (P), CD38, CD58, and CD19 can play a primary role from both diagnostic and prognosis point of view linked to pediatric cancer and therefore a better treatment protocols and more lives saving.

The CD10 found in 53.6% of patients in our study are as follows: 37% in B-cells and 16.6% in precursor B-cells. The CD34 are shown in about quarter of patients under research (26%), 16.5% in B-cells and 9.5% in precursor B-cells respectively. The results of CD20 (P) found in 29% of patients' files examined in our research are as follows: 23% in B-cells and 6% in precursor B-cells. The CD58 are found in 44% of patients; 27.4% in B-cells and 13% in precursor B-cells, whereas 2.4% in T-cells. The CD38 was appeared in 32% of patients; 24% in B-cells and 8% in precursor B-cells respectively. The CD19 found in our study is 54% of which a 37% is present in B-cells and 15.5% in precursor B-cells respectively. The markerCD7 was found positive in 6% of patients with T-cells and zero percent in patients with B-cells. As shown from our findings most of the markers were associated with either B-cells or precursor B-cells, and CD7 can consider as a marker of T-cell although its percentage was low.

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List of Acronyms and Abbreviations

ALL	Acute Lymphoblastic Leukemia
AIEOP	AssociazioneItaliana di EmatologiaedOncologiaPediatrica
CALL	Childhood Acute Lymphoblastic Leukemia
CD	Cluster of Differentiation
CBC	Complete Blood Count
CNS	Central Nervous System
CCG	Children's Cancer Group
CTEP/NCI	Cancer Therapy Evaluation Program of the National Cancer Institute
CI	Confidence Interval
EFS	Event Free Survivals
EGIL	European Group for the Immunological Characterization of Leukemia
EMR	Electronic Medical Record
FAB	French-American-British
FSC	Forward Scatter
FACS	Fluorescence Activated Cell Sorter
FISH	Fluorescence in Situ Hybridization
HgB	Hemoglobin
HLA-DR	Human Leukocyte Antigen-D Related
ITP	Immune Thrombocytopenia
IgG	Immunoglobulin
LAIS	Leukemic-Associated Immunophenotypes
MOH	Ministry of Health
MRD	Minimal Residual Disease

OS	Overall Survival
PLt	Platelets
PCR	Polymerase Chain Reaction
POG	Pediatric Oncology Group
PEG	Polyethylene glycol
SEER	Surveillance, Epidemiology, and End Results
SSC	Side Scatter
WBC	White Blood Cells

Chapter One:

Introduction

1.1 Background

Leukemia was reported as a set of blood and bone marrow cancers that threatens the life of patients (Hunger & Mullighan, 2015). It occurs when there is an abnormality in bone marrow, and leads to the production of many odd blood cells (Moschoi et al., 2016). It influences mostly people older than fifty five years, but it is also a common cancer in people younger than fifteen years old (Atkin et al., 2017). It could be a deadly disease; but nowadays there are many methods adopted to treat and control this illness and its related symptoms (Rose-Inman & Kuehl, 2017).

The most popular children cancers are: Childhood Acute Lymphoblastic Leukemia (CALL) (Bachir et al., 2009; Greaves, 2018) and Acute Lymphoblastic Leukemia (ALL). ALL is defined as malignant proliferation and transformation of progenitor lymphoid cells found in blood, bone marrow, and other extra modularly locations (KUMAR et al., 2019; Shafique & Tehsin, 2018; Terwilliger & Abdul-Hay, 2017).

The MRD is defined as a small number of leukemic cells which stay in a patient when they are in remission during or after treatment. It is the main reason of relapse in leukemia (Othus et al., 2020). The first report about MRD was issued four decades ago (Campana & Pui, 2017).

Knowledge of leukemic cells marker profile and technological advancements will lead to invent and improve flow cytometry technologies that can detect one leukemic cell out of ten thousand or more normal cells. The MRD levels assessment can be introduced at multiple phases of patients' therapy with ALL who are in complete remission with no morphological signs of illness, replacing standard microscopic approaches. Patients

with ALL have varying responses to the first therapeutic induction and the time it takes to reach MRD negative (Campana & Pui, 2017).

The MRD levels at the start of therapy provide reliable information regarding the sensitivity of drug dosages in leukemic lymphoblast; as a result of this relationship, MRD monitoring has become a key routine in the treatment of leukemia (Campana & Pui, 2017). MRD >1% increases the likelihood of relapse in children and adults with ALL; thus these patients are not treated with chemotherapy alone and are instead indicated for Hematopoietic Stem Cell Transplantation (Campana & Pui, 2017).

Flow cytometry with six or seven colors and PCR are two methods for detecting MRD, although flow cytometry is not as sensitive as PCR. Many modifications have been made to increase the sensitivity of flow cytometry, and eight colors immunostaining is an improvement of flow cytometry to detect MRD (Theunissen, Mejstrikova, Sedek, van der Sluijs-Gelling, et al., 2017).

It is critical to distinguish between leukemic cells and non-malignant cells of B-lymphocyte precursors when utilizing MRD analysis with flow cytometry to identify leukemic-associated immunophenotypes that exist at diagnosis. This is related to the recovery of bone marrow during chemotherapy stages (Rocha et al., 2016).

In ALL patients, Leukemia Associated Immunophenotyping (LAIP) is particularly significant in MRD assessment, since it is an important tool for identifying leukemic cell aberrations and leukemia typing depending on the source of leukemia as B-cell or T-cell precursor (Jalal et al., 2017).

Blast lineage identification is critical for determining illness prognosis, since it relies on the identification of immunological markers and cell antigens, as well as the detection of lineage and cell maturity. This information aids in the selection and improvement of

future treatments (DiGiuseppe & Wood, 2019). LAIP differs from well recognized patterns and must be recognized for assessing therapeutic effectiveness and detecting relapse (Garcia-Medina et al., 2014).

Characterizing the cells that cause Leukemia Immunophenotyping at diagnosis provides useful information for monitoring treatment, therapy dosages, and intensity by allowing the detection of remaining leukemic cells and illness classification based on the lineage and maturation of afflicted cells (Ribeiro, 2016). This emphasizes the need of assembling a comprehensive panel of monoclonal antibodies that will enable for the detection of abnormal antigen expression (Rocha et al., 2016). This includes antigen expression that occurs naturally at different stages of maturity, antigen expression from various cell lineages (myeloid, T-lymphoid, B-lymphoid), and antigen detection, such as low, excessive, or loss of expression. Blast cell antigen expression reflects the leukemic clone's genetic abnormalities (Rocha et al., 2016).

The MRD detection, or MRD positivity in ALL patients, is linked to poor Event Free Survival (EFS) and Overall Survival (OV). It indicates that if MRD exists or if you have positive MRD, your EFS and OV will suffer (Berry et al., 2017). In United States and European pediatrics trials, patients' risk is stratified depending on their MRD status, with intense therapy in the presence of MRD and lowering therapy in the absence of MRD (Berry et al., 2017).

All of the studies reviewed in the literature review agree on the need of MRD monitoring (Berry et al., 2017; Tahir, 2018).As a result, this study aims to determine the status of MRD in Palestinian children with ALL, analyze the results, and attempt to link the data received from the test result with treatment response, as well as define immunoglobulin markers in those Palestinian ALL children.

1.2 Study Justification

The MRD test is carried out on children with ALL who are being treated at the Palestinian public hospital in Beit Jala. The goal is to determine the significance of the MRD test and its influence on treatment protocols, as well as to determine the relationship between mutations in these patients and response to treatment. This is monitored starting with the first dosage of medication induction.

To the best of our knowledge, this is the first research of its kind in Palestine for children with ALL to see if the global procedures that connect the use of information received from MRD results with treatment strategies are being followed at the hospital under investigation (Beit Jala Hospital).

This study is critical for Palestine since it sheds light on children cancer, specifically MRD, a test that helps doctors select treatment methods. It will attempt to identify malignant immunophenotypes in Palestinian children with ALL, to establish association between these findings and therapeutic pathways, and to validate the value of incorporating the MRD test into protocols of therapy as a means of measuring the response to the first induction of chemotherapy.

In this study the researcher tries to connect the utilization of MRD test monitoring with the therapy protocols used by oncology specialists by evaluating the medical files of ALL patients treated in Beit Jala Hospital, which will then be assessed to find if these protocols are the best method to treat these patients.

This research will also reveal the immunophenotyping of malignant cells in Palestinian ALL patients, as well as the relationship between immunoglobulin markers and the patients' response to treatment.

1.3 Problem Statement

The cancer prevalent in Palestine is 80.3 new cancer cases per hundred thousand persons diagnosed each year(Ministry of Health, 2019).Lung cancer was the most prevalent cause of death among men and breast cancer among women, while leukemia was the most common among children(Testing & Cancers, 2020).

According to The Palestinian Ministry of Health (PMOH), 6.8% of these instances included children under the age of 15(Venkateswaran et al., 2018).

One of the challenges faced the physicians in controlling the disease and its repercussions is the time it takes to confirm a diagnosis of leukemia (Rose-Inman & Kuehl, 2017).

This study will be conducted to ensure the significance of MRD testing at the start of treatment, during treatment, and after the patient has reached remission. By incorporating this test into treatment protocols, doctors can prevent unnecessary severe chemotherapy and forecast relapse in some patients, as well as change the treatment regimen if necessary (Zhao et al., 2019). In addition, this study will identify the most common immunophenotypes among Palestinian patients and, if possible, link these abnormalities to the kind and length of treatment.

1.4 General Objective

The major goal of this study is to use 8-color flow cytometry to identify the immunophenotyping of ALL in Palestinian children, and to highlight the relevance of the MRD test in leukemia treatment as a crucial protocol of therapy.

1.5 Specific Objectives

The specific objectives are:

1. To identify the immunoglobulin markers found in ALL patients from Palestine.
2. Determine the importance of MRD monitoring in determining the treatment plan.
3. To find if there's a link between blood components as WBC, HGB, and platelet count and leukemia diagnosis.
4. To determine whether there is a link between the discovery of immunophenotypes and the response to the treatment offered to these patients.
5. Use the data from this test to investigate patient immunophenotyping, such as the important markers found in patients treated at Beit Jala Hospital, and to see if there is a link between these markers and therapy response, as well as any link between the presence of specific markers and relapse, if it occurs.

1.6 Expected Outcomes

During this investigation, the researcher will strive to identify the leukemia-linked immunophenotypes that could lead to ALL in Palestinian youngsters, as well as link these alterations to treatment response and relapse if it occurs.

Chapter Two:

Literature Review

2.1 Background

ALL is a cancer that is growing fast and affecting white blood cells called lymphoblasts. It is a malignant clonal proliferation of lymphoid progenitor cells that develop into subtypes B and T, as shown in figure 1 (Board, 2020). These cells are typically present in the bone-marrow, which produces a significant number of immature lymphoblasts, hinder good blood cells from maturing, and do not operate properly to fight infection (Vadillo et al., 2018).

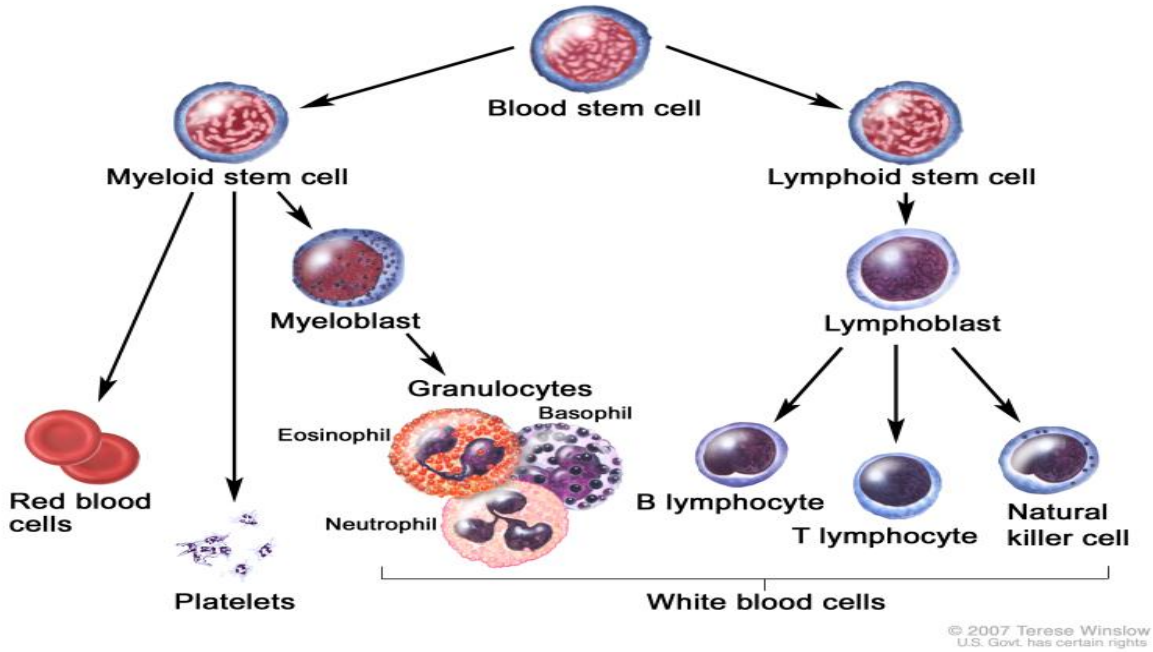

Figure 1: Development of Blood cell (Board 2020).

2.2 Acute Lymphoblastic Leukemia epidemiology

In The United States (US), over 3000 children are diagnosed with ALL yearly, with an annual incidence of 3.7-4.9 cases per 100,000 children aged 0 to 14, and a comparable

projected worldwide incidence (Board, 2021). Although the total prevalence of childhood cancer, including ALL, has been slowly increasing since 1975, cancer in children and adolescents is uncommon (Steliarova-Foucher et al., 2018). Children and adolescents with cancer have seen dramatic increases in survival rates. Between 1975 and 2010, the death rate from pediatric cancer fell by more than half (Kassebaum et al., 2017).Over the same time period, the 5-year survival rate for ALL children younger than 15 years was increased from 60% to almost 90%, and for adolescents aged 15 to 19 was increased from 28% to more than 75% (Masquelier et al., 2018). Cancer therapy side effects may persist or develop months or years after treatment in children and adolescents, necessitating constant monitoring (Chang et al., 2017). Overall survival rates for children with Acute Lymphoblastic Leukemia have approached 90% with the advances in diagnosis and therapy (Board, 2021).

According to the studies below, it appears that the incidence of childhood leukemia is on the rise. According to the Surveillance, Epidemiology, and End Results (SEER) database, ALL occurrences increased steadily from the year 1975 till 2010, with an increase of around 0.7 yearly (Noone et al., 2017). There is a twenty-five cases increase per million in 1975 to thirty-four cases per million in 2010 (Ward et al., 2014).

Data collected from 63 European population-based cancer registries of children diagnosed with malignancy shows the incidence of leukemia increased 1.4 percent during the years 1970-1999 (Autier, 2018). The incidence of leukemia including ALL was increased by 1.4 percent from 1971 to 2000. The incidence of ALL-related leukemia in the United Kingdom consistently increased from 3.83 to 4.61 per 100,000 people, depending on sex and age (Rachet et al., 2009).

In contrast, a study from four Nordic nations (Denmark, Finland, Norway, and Sweden) found that the incidence of pediatric ALL remained steady between 1983 and 2002 with around 3.3 cases per 100,000 children under the age of 15 (Shah & Coleman, 2007). Figure 2 shows trends in childhood ALL incidence rates by race/ethnicity from 1992 to 2013. Childhood ALL incidences increased among Hispanic White children but not among non-Hispanic children; however, the difference in trends was not statistically significant (APC P interaction= 0.29) (Barrington-Trimis et al. 2017).

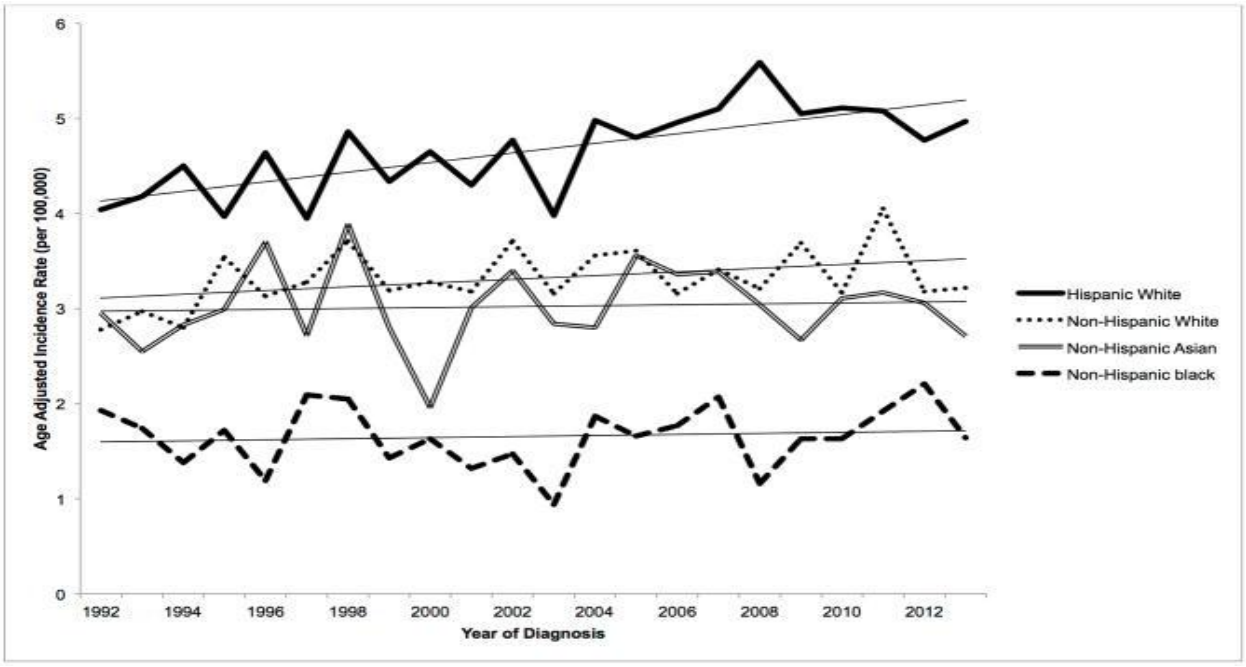

Figure 2: Pediatric All Incidence Rates by Race and Ethnicity, SEER 13, 1992–2013.

According to (Bailony et al., 2011) there were elevated rates for childhood cancers between 1998-1999; leukemia and lymphoid leukemia elevated in 2006, statistically significant periods with low rates were found for leukemia in 2003-2005, and lymphoid leukemia as well as in 2003-2005

2.3 Clinical Symptoms and Signs of ALL at Early Stages

Nonspecific symptoms as weight loss and lack of appetite, night sweats, fever, bleeding, bone pain, and Lymphadenopathy are the most common presenting signs of ALL(Coluzzi et al., 2020). Any of these typical signs or symptoms that aren't explained should be taken seriously as a sign of malignancy.

The following are the most familiar presenting signs or symptoms of leukemia that indicate malignancy (Coluzzi et al., 2020):

1. Musculoskeletal discomfort: Any child who has bone pain and peripheral blood abnormalities should have a bone marrow test done as soon as possible. Leukemic involvement of the periosteum causes bone discomfort, particularly in the long bones.
2. Headache: Although it is rare (< 5% of the time), leukemia of the central nervous system (CNS) can cause symptoms of elevated intracranial pressure, such as nuchal rigidity, vomiting, headache, and lethargy.
3. Lymphadenopathy: Malignancy-related Lymphadenopathy is usually non-tender, hard, rubbery, and matted.
4. Testicular enlargement: Unilateral testicular enlargement with no pain can be a presenting indication of ALL, although it is unusual.
5. Mediastinal mass: T-cell ALL is most common in older boys (older than or are 10 years old) with a large mediastinal mass and high initial WBC counts (50,000/ul) (Verma et al., 2020).
6. Blood abnormalities in the peripheral circulation: Most children with ALL exhibit anemia, thrombocytopenia, and regular or slightly elevated white blood cells counts or lymphoblasts on a peripheral smear (Horton et al., 2018).

2.4 Classical Lab Tests and Clinical Procedures for ALL

ALL may manifest as a large lesion or leukemia in the clinic. When the bone marrow includes more than 25% lymphoblast, ALL is the preferable name; whereas lymphoma is the optimum term when the pathology is limited to a mass lesion with minimum or no blood and bone marrow involvement (Alvarnas et al., 2015).

Leukemia is diagnosed and classified using specialized tests done on cells generated from a tissue biopsy or bone marrow aspirate specimens. When bone marrow testing is not possible, cells from pleural effusions or peripheral blood can be used to diagnose ALL and determine if leukemia cells have moved to other regions of the body, such as the brain or testicles (Bain, 2017).

Bruising, hepatosplenomegaly, swollen lymph nodes, and petechiae or purpura are all things that a physical exam can detect. Also, the number of platelets, RBC, the type and number of WBCs, the amount of HB in RBCs, and the percentage of the sample made up of red blood cells are all measured in the Complete Blood Count (CBC) test that can be used for diagnosis. Blood chemistry tests are utilized to specify the number of specific compounds released into the bloodstream by the body's tissues and organs. A high or low concentration of chemistry might be considered as a symptom of sickness. In addition, aspiration of bone marrow for flow cytometry biopsy and diagnosis of leukemic cells for morphological diagnosis can be used (Clarke et al., 2016).

Immunophenotyping and molecular cytogenetic testing are two techniques used to define and stratify leukemic cells. Markers on the surface of bone marrow cells and antigens are used in immunophenotyping to classify lymphoid and myeloid cells, and if the cells are malignant lymphocytes, these antigens are utilized to classify them as B lymphocytes or T lymphocytes (Chiaretti et al., 2014).

Under a microscope, molecular cytogenetic analysis is utilized to characterize the alterations in chromosomes in lymphocytes. The replacement of a part of one chromosome on another chromosome in Philadelphia chromosome is a positive ALL (Mrózek et al., 2009).

Another test is Fluorescence in Situ Hybridization (FISH) that can be used to determine specific chromosomal alterations. Furthermore, various tests such as lumbar puncture, chest X-ray, and testicular biopsy are performed to detect leukemic cell relapse and metastasis (Tomizawa & Kiyokawa, 2017).

2.5 Differential Diagnosis

Because the existing signs and symptoms are nonspecific, a separate malignant and nonmalignant condition must be addressed in the differential diagnosis of ALL. They include the following: " *Juvenile idiopathic arthritis, Osteomyelitis, Epstein-Barr virus, Immune thrombocytopenia (ITP), Aplastic anemia, acute infectious lymphocytosis, other malignancies with bone marrow involvement (neuroblastoma, retinoblastoma, rhabdomyosarcoma, and Ewing sarcoma), and Hyper eosinophilic syndrome*''(Emmi et al., 2014).

2.6 Features of Pathological ALL

ALL has traditionally been diagnosed visually using the French-American-British (FAB) classification, which incorporates information from the bone marrow aspirate about the size, volume of cytoplasm, and relevance of nucleoli of tumor cells (Bain, 2017).

Despite the fact that some doctors continue to utilize this approach to classify and explain the phenotypic of tumor cells, it has lost its predictive utility as our understanding of disease biology has increased and treatment regimens have become more intensive and successful. The FAB criterion, on the other hand, is not currently used in diagnosis or therapy decisions (Bain, 2017; Clarke et al., 2016).

As shown in Figure 3 (Kröber et al., 2000), L1 lymphoblasts are small cells with poor cytoplasm, compacted nuclear chromatin, and indistinct nucleoli. FAB L1 is present in almost 85 to 89 percent of pediatric ALL cases.

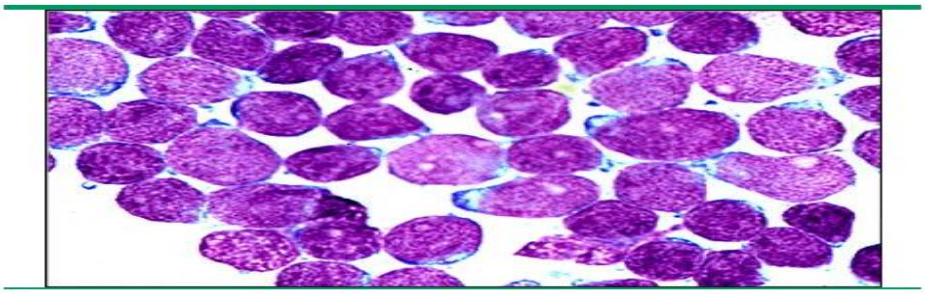

Figure 3: L1 morphology in a bone marrow (Kröber et al. 2000).

As seen in Figure 4, L2 lymphoblasts are bigger cells with a considerable quantity of cytoplasm, scattered chromatin, and many nucleoli(Wahhab, 2015). The L2 has been linked to a worse prognosis than L1 in various studies. When patients are stratified by age, sex, and starting WBC, there are no longer any differences in prognosis between L1 and L2. FAB L2 is the most common type of pediatric ALL, accounting for 11 to 14% of all cases.

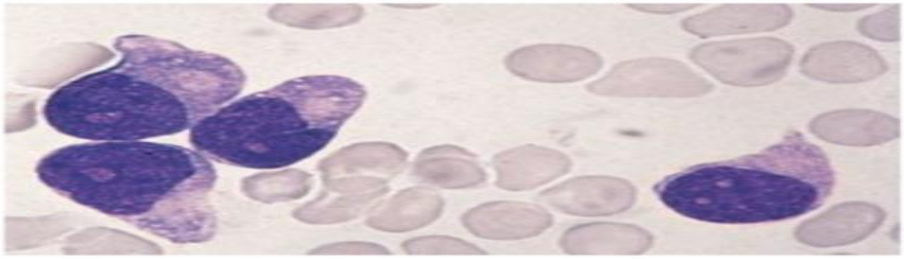

Figure 4: L2 morphology of a patient's bone marrow from an ALL patient (Wahhab 2015).

As demonstrated in figure 5, L3 lymphoblasts feature deep cytoplasmic basophilia with prominent cytoplasmic vacuolation. The L3 morphology is linked to a more cautious outlook. However, FAB L3 is only seen in about 1% of all pediatric ALL cases.

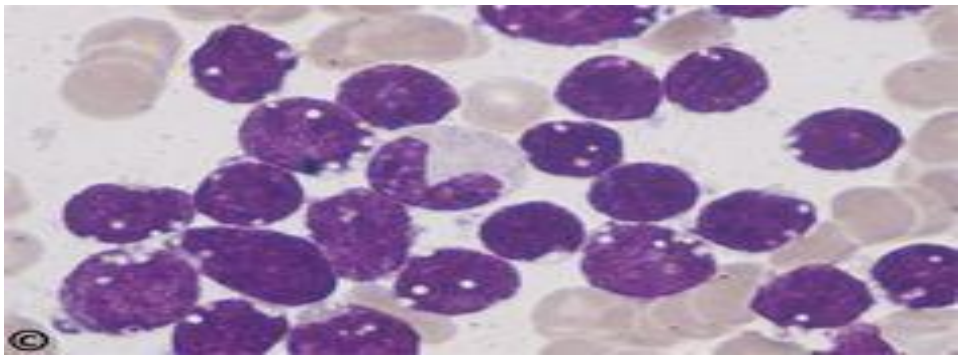

Figure 5: L3 morphology of a patient with ALL's bone marrow aspirate

2.7 ALL Immunophenotyping

The World Health Organization (WHO) has changed the FAB categorization of ALL based on Immunophenotyping, utilizing a large panel of monoclonal antibodies to cell surface "cluster of differentiation" (CD) markers (Wohlfahrt et al., 2015).

Multi-channel flow cytometry immunophenotyping has become the standard approach for ALL diagnosis and sub-classification(Jaafar & Kadhom, 2018). It was also designed to be a helpful tool for detecting and monitoring MRD. The European Group for the Immunological Characterization of Leukemia (EGIL) has agreed that a threshold of 20% should be used to define a positive reaction of blast cells to a given monoclonal antibody (Jaafar & Kadhom, 2018).

The main ALL immunological subtypes identified are: precursor B-cell ALL (about 80% of cases), mature B-cell ALL (about 2-5 percent of cases), precursor T-cell ALL (about 15-17 percent of cases), and mature T-cell ALL (about 15-17 percent of cases). These were the four main ALL immunological subtypes identified (Teachey & Pui, 2019). If the cells display cytoplasmic CD79a and immunoglobulin (IgG) as well as

surface CD19, CD10, HLA-DR, CD34, CD20, CD22, and nTdT, the case is classified as precursor B-cell ALL. If the cells also express surface IgG and have clonal lambda or kappa light chains while being negative for nTdT and CD34, the case is classified as mature B-cell ALL(Boer et al., 2016).

If the cells display cytoplasmic CD3 and surface CD7, CD1a, CD2, CD5, CD4, CD8, CD34, and nTdT, the case is classified as precursor T-cell ALL. If the cells additionally express surface CD3, CD4, or CD8, and are negative for CD1a, CD34, and nTdT, it is termed mature T-cell ALL (Jalal et al., 2017). The CD10 expression is widespread (25 percent) and non-specific in T-cell ALL (Jalal et al., 2017). In addition to their unique lymphocyte markers, certain B-cell and T-cell ALL express myeloid markers such as CD13 and CD33 (Jalal et al., 2017).

2.8 ALL Cytogenetic

In the risk classification of pediatric ALL, cytogenetic characteristics are very relevant. Gene rearrangements and fusions affect patient prognosis by affecting both chromosome number and chromosome shape (Karrman & Johansson, 2017).

2.8.1 Numeric abnormalities in ALL

Ploidy alterations, as well as the acquisition or loss of individual chromosomes, can be influenced by numerical anomalies (aneuploidy changes). In patients with ALL, the following chromosomal number anomalies are prognostic indicators:

- High hyperdiploidy (50 or more chromosomes) is a favorable prognostic factor that has been consistently linked to a favorable outcome in ALL cancers (Pui et al., 2015). Patients with substantial hyperdiploidy, such as double trisomies of

chromosomes 4 and 10, or triple trisomies of chromosomes 4, 10 and 17, have the best outcomes in clinical trials conducted in the United States (Cooper & Brown, 2015).

- Hyperdiploidy (having fewer than 46 chromosomes) is a bad predictor of prognosis. With a drop in chromosomal numbers, the likelihood of a negative outcome increases. Hypodiploid instances with only one chromosome, on the other hand, have a comparable prognosis to diploid cases, and near-haploid cases (24 to 28 chromosomes) have the worst prognosis (Pui et al., 2015).
- Although near triploidy (68 to 80 chromosomes) or near tetraploidy (greater than 80 chromosomes) is associated with a poor prognosis in most cases, a significant series of B-cell lineage cases has indicated a favorable prognosis(Chiaretti et al., 2014).

2.8.2 Structural Abnormalities in ALL

Translocations, deletions, insertions, and inversions are examples of structural gene abnormalities. In patients with ALL, the following structural chromosomal anomalies are prognostic indications (Elkarhat et al., 2019):

- The TEL-AML1 fusion gene is produced by the t (12; 21) translocation. A favorable prognosis is linked to this structural alteration.
- One of two structural rearrangements linked to poor prognosis is the t (9; 22) translocation, also known as the Philadelphia chromosome.
- The MLL gene rearrangement at 11q23 has also been linked to a bad prognosis.

2.9 Risk Stratification and Factors Related to Prognosis Currently Used to Direct Therapy in ALL

Many clinical and biochemical variables have been found to be prognostic in the clinical prognosis of ALL patients. Patients' age, gender, WBC count at diagnosis, and immunophenotyping pattern are all considered as conventional features, with age at diagnosis and initial WBC count being the most important prognostic markers (Kato & Manabe, 2018).

Infants under the age of one, teenagers over the age of nine, and pediatricians with a WBC count of more than 50,000/l are all at danger. This is because T-cell and mature B-cell immunophenotypes are linked with a very poor prognosis, whereas precursor B-cell immunophenotypes are associated with a good prognosis. Lymphoblast immunophenotyping is a highly significant predictive characteristic, as shown in Table 1 (Von Stumm & Plomin, 2015):

Table 1: Prognostic variables in juvenile acute lymphoblastic leukemia (Von Stumm & Plomin, 2015).

Risk Factor	Favorable Outcome	Unfavorable Outcome
Age	>1and<10years	<1or>10years
Gender	Female	Male
WBC count at diagnosis	<50,000/ul	>50,000/ul
Immunophenotypes	Precursor B-cell	T-cell, or mature B-cell

The prognostic usefulness of sex is debatable, since some studies show no difference in prognosis between males and females, while others, particularly in the United Kingdom (UK) and Nordic countries, have discovered that males have a much greater relapse rate than females (Helgadottir, 2015).

The majority of prognostic markers used to decide the degree of therapy nowadays is clinical or biological in nature, and can be evaluated at the time of diagnosis. The major clinical trial groups treating pediatric cancer in the United States use a wide range of risk stratification methods for ALL. ALL in mature B-cells is handled differently than ALL in precursor B-cells and T-cells. The treatment of precursor B-cell ALL, and occasionally T-cell ALL, is largely determined by the patient's age and white blood cells count at diagnosis time as shown in Table 2 (Zeidan et al., 2017).

Table 2: outcome of 4-years event free survival for children with precursor B- cell

	Age(years)			
White blood cells count/ul:	**1-2.99**	**3-5.99**	**6-9.99**	**≥10**
<10,000				
4-year event free survival%	82.9	84.7	82	69.6
Number of patients treated	490	937	437	406
%precursor B-cell patients	10.7	20.5	9.6	8.9
10,000-49,999				
4-year event free survival%	74.6	74.5	80.2	59.2
Number of patients treated	436	608	205	236
%precursor B-cell patients	9.5	13.3	4.5	5.2
≥50,000				
4-year event free survival%	68.3	73.9	47.5	41.1
Number of patients treated	278	280	122	140
% precursor or B-cell patients	6.1	6.1	2.7	3.1

However, because of inconsistencies in the definitions of treatment-related risk categories, comparing the results of different clinical tests has been challenging. As a result, the National Cancer Institute's Cancer Therapy Evaluation Program (CTEP/NCI) hosted a workshop in 1993 to try to develop a standardized strategy to risk classification

in order to improve clinical research efficiency. Patients with precursor B-cell ALL, aged one to nine years, with a WBC count less than 50,000/l at diagnosis, which account for the majority of individuals, are at lower risk than other patients, according to the collaborating scientists (Karrman & Johansson, 2017). Table 3 depicts POG and CCG patients' four-year event-free survival when comparing the standard risk group to the greater risk group at the time of the workshop (Tomizawa & Kiyokawa, 2017).

Table 3: uniform age WBC criteria for precursor B-cell ALL

Risk	Definition	4-yeareventfree Survival%	%ofprecursorB-cell Patients
Standard	WBC count<50,000/ul and age 1-9.99years	80.3	68
High	WBCcount≥50,000/ul or age ≥10years	63.9	32

Nonetheless, modern stratification methods use these parameters, as well as a variety of other characteristics, to divide patients into treatment groups, such as immunophenotyping by age and WBC count. T-cell ALL patients, for example, are often older than precursor B-cell ALL patients, which helps to explain why older people are at such a high risk. A higher WBC count is also associated with a bad prognosis in elderly patients (Tomizawa & Kiyokawa, 2017).

Extramedullary illness, such as those found in the CNS, is also considered in determining therapy intensity. T-cell ALL has a higher prevalence of CNS leukemia than precursor B-cell ALL, which is diagnosed in less than 5% of children and is associated with a poor treatment prognosis (Garcia- Medina et al., 2015). More than five white blood cells per milliliter of spinal fluid that are morphologically blasts are considered CNS illness.

Other characteristics considered include: male, older than nine years old, high WBC count at diagnosis, poor CD10 expression, a mediastinal mass, and having CNS involvement (Wahhab, 2015).

2.10 Pediatric ALL Treatment

Multiple factors, including a better understanding of the immune-biology of ALL and disease burden, recognition of shelter sites and integration of pre-symptomatic central nervous system prophylaxis, lineation of prognostic factors with risk-adapted treatment, and improvements in supportive care, all have contributed to improvements in the 5-year survival rate for pediatric ALL. Overall, more than 95 percent of patients will achieve remission, and close to 85 percent will live for at least 5 years without a leukemic recurrence after diagnosis (Zeidan et al., 2017).

One of the hallmarks of pediatric ALL treatment is the reliance on risk-based classification. Patients can be categorized into groups based on the probability of treatment failure by determining clinical and biochemical factors that have been found to affect prognosis. Those with positive clinical and biological features can be treated with a low-dose regimen to avoid toxicity, whereas those with higher-risk disease can only be treated with more aggressive regimens to optimize cure. As demonstrated in Table 4, it is critical to identify those characteristics that have been shown to consistently influence prognosis and treatment (Karol & Pui, 2020).

Children ALL requires a multidrug regimen organized into three phases (induction, consolidation, and maintenance), as well as CNS therapy. Most treatment plans, on the other hand, take two to three years to finish (Cooper & Brown, 2015).

Table 4: Clinical risk assignment and suggested therapies in pediatric ALL

Risk Group	Features	Percent%	Recommended Therapy
Low	Hyperdiploidortrisomies4,10,17	20	Conventional anti-metabolite-based therapy
	T(12,21)	20	
Standard	WBC<50,000/micro/L	15	Intensified anti metabolite therapy
	Age1to 9.9years		
High	T-cell Phenotype	15	Intensive multi-agent therapy
	Age>10years	15	
	WBC>50,000/micro, t(1;19)	6	
Very High	t(9;22)	3	Consider allogenic hematopoietic cell Transplantation in first remission
	t(4;11);age<1year	4	
	Induction failures and slow responders	2	

2.10.1 Induction Therapy of ALL

Induction therapy is the first block of chemotherapy and lasts 4 to 6 weeks, with the goal of putting the patient in complete remission and restoring normal bone marrow hematopoiesis. Regardless of their baseline risk categorization, more than 90% of ALL children and adolescents achieve full remission by the completion of induction therapy (Schrappe et al., 2012).

Clinical prognosis is best predicted by early lymphoblast clearance from the bone marrow, the presence of MRD on day 15, and the conclusion of induction therapy on day 33. Patients who respond fast to the induction regimen appear to have a better prognosis than those who respond slowly or lose induction therapy (Garcia- Medina et al., 2015).

Vincristine, corticosteroids, and asparaginase are used in the induction phase, with an anthracycline such as doxorubicin or daunorubicin added to most regimens. However, randomized trials have demonstrated that both anthracyclines have comparable efficacy and toxicity. In order to reduce toxicity, several organizations avoid giving anthracyclines to people who are at a lesser risk. Prednisone or dexamethasone are commonly used corticosteroids, with dexamethasone having better CNS penetration and a lower chance of relapse, but with an increased risk of toxicities include vascular necrosis, infection, and linear growth loss (Cooper & Brown, 2015).

Polyethylene glycol (PEG) asparaginase and Erwiniaasparaginase are two agents for asparagine depletion. In comparison to native Escherichia coli L-asparaginase, PEG asparaginase has been changed by covalently attaching polyethylene glycol, resulting in a longer half-life and lower immunogenicity. The pegylated version, on the other hand, has been demonstrated to be more effective in randomized studies. Erwiniaasparaginase, on the other hand, is frequently administered to individuals who have had an allergic reaction to PEG asparaginase, and therefore necessitates a more regular delivery regimen (Heo et al., 2019).

2.10.2 CNS Preventive Therapy

CNS involvement in individuals with leukemia at the time of diagnosis is a rare occurrence, occurring in less than 5% of cases (Faderl & Kantarjian, 2018). Despite the fact that systemic chemotherapy could induce bone marrow remission, most children subsequently had CNS relapse in the absence of targeted therapy. This strategy comprises both therapy and prophylaxis for patients with clinical CNS illness at the time of diagnosis (Heo et al., 2019).

Direct intrathecal chemotherapy, systemic chemotherapy capable of penetrating the blood-brain barrier, and cranial radiation are all options for eradicating illness from the CNS. During remission induction, all treatment options include intrathecal chemotherapy injection. Intrathecal treatment is included in some protocols throughout treatment, but not in others (Faderl & Kantarjian, 2018).

Intrathecal methotrexate or a triple intrathecal therapy including intrathecal methotrexate, cytarabine, and hydrocortisone are two possibilities for intrathecal chemotherapy. Although some data suggests that triple intrathecal therapy reduces the probability of CNS relapse, studies have found no meaningful difference in overall or event-free survival. Dexamethasone, high-dose methotrexate, 6-mercaptopurine, cytarabine or cyclophosphamide, and L-asparaginase are examples of systemically administered chemotherapy having CNS effects (Faderl & Kantarjian, 2018; Heo et al., 2019).

2.10.3 Consolidation Therapy of ALL

The second phase of ALL treatment, known as consolidation or intensification therapy, begins soon after complete remission is achieved. Small numbers of leukemic lymphoblasts known as MRD remain exist in the bone marrow despite histological verification of complete remission after induction therapy, necessitating further treatment to remove the submicroscopic residual illness. If therapy is not continued in these circumstances, relapse happens fast (Heo et al., 2019). The purpose of post-induction chemotherapy is to limit leukemic regrowth, minimize burden of residual tumor, and prevent drug resistance from developing in residual leukemic cells (Garcia-Medina et al., 2015).

Intensification of therapy regimens lasts about 6 to 9 months and is based on the patient's risk of a bad outcome. With the purpose of enhancing survival, intensified therapy is reduced for patients with a good prognosis while a more intensive treatment is delivered to those at high risk (Faderl & Kantarjian, 2018; Garcia- Medina et al., 2015). Mercaptopurine, thioguanine, methotrexate, cyclophosphamide, etoposide, and cytarabine are commonly used in this phase of chemotherapy to optimize synergy and decrease drug resistance (Heo et al., 2019).

2.10.4 Maintenance Therapy of ALL

The third, last, and longest step of treatment for pediatric ALL is maintenance or continuation chemotherapy. Once remission has been established after completion of the consolidation or intensification phase of therapy, a far less rigorous regimen and a longer maintenance phase have been shown to reduce the chance of relapse. The goal is to eliminate any leftover leukemia cells that could re-grow and induce a relapse (Faderl & Kantarjian, 2018; Heo et al., 2019).

Antimetabolite therapy with methotrexate and mercaptopurine, both of which come in oral forms, is the cornerstone of maintenance therapy. Although the evidence for further benefit is questionable(Heo et al., 2019), some regimens also include monthly vincristine and steroids (Faderl & Kantarjian, 2018).

2.11 Difficulties in Defining Complete Remission in ALL

The purpose of induction therapy of ALL is to achieve an initial complete remission, which is defined as the removal of all leukemic cells (less than 5% of blasts) from the bone marrow and blood that can be detected by microscopic review, as well as the restoration of normal hematopoiesis (more than 25% cellularity and normal peripheral

blood counts) (Cave & van der Werff ten Bosch, 1998; Heo et al., 2019). The same source also hypothesized that an assessment of MRD would define a more difficult (full MRD response) that could be better able to forecast prognosis. Many observations corroborate this approach, explaining the difficulties in determining whether a patient with ALL in morphologic full remission would remain disease free (Cave & van der Werff ten Bosch, 1998):

1. Hematogones: Morphologic examination may be unable to distinguish ALL blast cells from lymphoid precursors (hematogones) or activated mature lymphocytes. In samples of bone marrow recuperating from chemotherapy or transplantation, when hematogones may make up 10% of the lymphoid cells, this uniqueness is extremely hard to achieve.
2. Sampling mistake: Because a single bone marrow samples represents such a small percentage of the entire cellular population of the bone marrow, sampling error is a possibility. However, there have been several documented cases where a bone marrow aspiration was normal in one location but revealed leukemia in another.
3. Detection limitations: Operator error and the number of metaphases analyzed limit the detection of ALL blasts by morphologic evaluation or traditional cytogenetic.

2.12 Minimal Residual Disease in ALL

Due to the poor sensitivity and specificity of morphological evaluation of bone marrow aspirates in the past, failure to detect remaining leukemic cells could result in under treatment and an increased risk of relapse. Misclassification of normal cells, such as

ALL blasts, on the other hand, could lead to overtreatment and a higher risk of treatment-related morbidity (Percival et al., 2017).

The therapy of ALL in children has progressed dramatically over the last three decades. Although current treatment options achieve long-term remission in roughly 80 percent of children with ALL, the remaining 20 percent relapse with a cure probability of about 25 to 40 percent after recurrence. Furthermore, some subgroups of children who are now receiving intense therapy may be over-treated and could be healed with less rigorous regimens, resulting in less toxicity and fewer long-term negative effects (Hefazi & Litzow, 2018).

In addition to risk factors linked with the patient, such as gender and age at diagnosis, and risk factors linked with the disease, such as white blood cells count at diagnosis and immunophenotyping, in vivo treatment effectiveness measurement has been confirmed to be the most important in predicting patient clinical outcome and relapse risk. Immunological or molecular markers, fluorescence in situ hybridization, in vitro drug response, and colony assays have all been developed to refine morphology in measuring treatment response. This technological innovation prompted the introduction of the MRD idea, which has called into question the traditional notion of remission (Hefazi & Litzow, 2018).

MRD is defined as the existence of leukemic cells below the detection threshold of traditional morphologic methods. It is an important component of patient evaluation and a powerful predictor of clinical success during sequential therapy in children with ALL (Kruse et al. 2020). The MRD readings during and after initial induction therapy have been shown to have a substantial link with the likelihood of relapse in children with ALL (Campana, 2009).

MRD assessment is most important after first induction, although other time periods may be relevant depending on the treatment plan. However, to establish MRD negativity, the assay sensitivity should be less than 0.01 percent, and multicolor flow cytometry and PCR techniques can detect leukemic cells with a sensitivity threshold of less than 0.01 percent bone marrow mononuclear cells (Campana, 2009; Kruse et al., 2020). Positivity of MRD is defined as the existence of 0.01 percent or more ALL cells; the risk of relapse is proportional to the level of MRD, especially when assessed during or after remission induction therapy. MRD, on the other hand, is a powerful independent prognostic marker that can indicate poor responders among AML patients (Campana, 2009).

Following induction chemotherapy, the vast majority of newly diagnosed ALL patients achieve complete morphologic and cytogenetic remission. While the majority of children live for a long time without relapse, some will develop leukemia and die. Relapse is caused by residual leukemic cells that remain after complete remission but are too small to be detected using traditional morphologic methods. MRD refers to subclinical levels of residual leukemia that can be assessed using more sensitive tests (Forman & Rowe, 2013). Complete remission was define as the absence of visible indications of leukemia or detectable leukemia cells on blood smears, active hematopoiesis in the bone marrow, and less than 5% leukemia blast cells, as well as normal cerebrospinal fluid. The International Berlin-Frankfurt-Munster Study Group (I-BFM-SG) has pioneered the assessment of MRD at days 33 and 78 of treatment as time points 1 and 2 to stratify patients into low, intermediate, or high-risk groups (dxalal, 2019).

Time point 1 appeared to be particularly beneficial for identifying low and intermediate

risk patients, whereas time point 2 appeared to be more useful for identifying high risk patients in this risk group classification. To identify the different response groups and differences in overall treatment response within T-ALL and precursor B-ALL, the BFM–Association Italiana di Ematologiaed Oncologia Pediatrica (AIEOP) uses MRD-based risk group classification for treatment stratification in pediatric ALL protocols (Theunissen, Mejstrikova, Sedek, Van Der Sluijs-Gelling, et al., 2017). However, the most favorable prognostic factor was MRD negative at time point 1 (day 33) (Schrappe et al., 2011).

2.13 Minimal Residual Disease Detecting Methods

Cytogenetic, cell culture methods, FISH, Southern blotting, multicolor flow cytometry, and Polymerase Chain Reaction have all been investigated for the detection of residual disease (Fang et al., 2012).

ALL MRD assays should be able to detect one leukemic cell out of 10,000 or more normal cells. They should also be able to distinguish between leukemic and non-leukemic cells with high accuracy and provide data in a timely manner. Finally, they should be reliable enough to produce consistent MRD estimations across laboratories. Flow cytometric analysis of leukemia-related immunophenotypes, as well as polymerase chain reaction amplification of antigen receptor gene rearrangements, are currently the most accurate approaches for studying MRD in ALL (Fang et al., 2012).

2.13.1 Multi color Flow Cytometry

A laser is used in multicolor flow cytometry to assess specific immunophenotypic traits of millions of cells per second. Flow cytometry can be used to detect MRD because

antigen expression is erratic(Bain, 2018).

The power of multicolor flow cytometry in determining MRD is shown in leukemic blasts in B-cell lineage ALL almost often co-express CD10, CD19, CD20, CD22, CD34, nTdT, and CD58, as well as some T-cell antigens like CD5 and CD7 and myeloid antigens like CD13 and CD33(Chiaretti et al., 2014). Terminal transferase, CD2, cytoplasmic CD3 (cCD3), CD5, nTdT, CD34, and CD7 are nearly always co-expressed in T-cell lineage ALL leukemic blasts (Cannizzo et al. 2011).

Sequential MRD monitoring with a multi-parameter flow cytometry approach has been demonstrated to be a useful predictor of relapse in children with ALL(Bain, 2018; Cannizzo et al., 2011). For example, in one study by (Chiaretti et al., 2014), those who were flow cytometry negative for MRD had a 10% cumulative rate of recurrence, whereas those with MRD of 0.1, 0.1 to 1.0, and 1.0 percent had 23, 43, and 72 percent cumulative rates of relapse, respectively(Othus et al., 2020).

2.13.1.1 Advantages of Flow Cytometry Technique

The following are some of the benefits of using flow cytometry to detect MRD(Jaafar & Kadhom, 2018):

1. Broad applicability: immunophenotyping using flow cytometry can be used to diagnose 80-95 percent of ALL patients.
2. Reliability: Results are reported the same day.
3. Quantitatively: Although not yet standardized, the results of flow cytometry are quantitative rather than qualitative.
4. Providing extra information about the malignant and benign cells in the sample that may aid drug targeting

5. The sample source can be bone marrow or entire blood.

2.13.1.2 Limitations of Flow Cytometry Technique

The following are some limitations of flow cytometry for MRD assessment (Bain, 2018):

1. Hematogones: Similar to leukemic blasts, low-frequency normal hematopoietic progenitor cells (hematogones) may express the same cytoplasmic or surface-bound marker profile, making differentiation between malignant and normal cells challenging in this scenario.
2. Immunophenotypic shifts: As the disease progresses, there's a chance that the leukemic cells' immunophenotypic expression will change, leading to a false negative result.
3. Bone marrow samples with low cellularity during and after induction treatment.

2.13.2 Polymerase Chain Reaction (PCR)

PCR can detect one malignant cell among 104 to 105 normal cells by amplifying a DNA or complementary DNA (cDNA) sequence specific to the leukemic clone (Theunissen, Mejstrikova, Sedek, Van Der Sluijs-Gelling, et al., 2017). There are two types of targets used to identify MRD in ALL patients:

1. Rearrangements in the genes for immunoglobulin (IgG) or T-cell receptor (TCR).
2. Chromosomal rearrangements unique to leukemia.

2.13.2.1 The advantages of PCR Technique for MRD Monitoring

The following are benefits of PCR for MRD monitoring (Tomizawa & Kiyokawa, 2017):

1. High sensitivity: While sensitivity varies depending on the junctional region target, PCR sensitivity is typically 0.5 to 1.0 log higher than flow cytometry.
2. Broad applicability: Given the high incidence of IgG and TCR gene rearrangements in ALL, PCR testing is feasible in the majority of patients.
3. Speed: A single DNA molecule can be amplified a million times in just a few hours using PCR.
4. Sample stability: PCR-tested DNA samples are relatively stable throughout travel.
5. Requirements for tissue are minimal.
6. A process that is standardized.

2.13.2.2 Limitations of PCR Technique for MRD Monitoring

According to (Theunissen, Mejstrikova, Sedek, Van Der Sluijs-Gelling, et al., 2017; Tomizawa & Kiyokawa, 2017), the downsides of PCR for MRD monitoring include a number of technical and biological concerns which are:

1. Contamination of the reaction product, which necessitates stringent quality assurance.
2. When there are a small number of transcripts, the repeatability is poor.
3. Leukemic clone evolution, sub clone creation, and/or the presence of oligoclonal populations, which can result in both false-negative and false-positive outcomes.
4. Diagnostic sample required: Because the junctional region rearrangements are unique to each leukemia clone, a sample from the time of diagnosis is required to

identify the appropriate primers.

5. Only experienced molecular hematology labs are permitted to participate.

2.13.3 Response Defined by Using MRD

In addition to standardizing the methodologies for assessing and quantifying MRD, practitioners and researchers should adopt consistent nomenclature to discuss individual patient findings and compare trial outcomes. As a result, (Schrappe et al., 2011), proposed the following definitions based on a panel of representatives from key European study groups on children and adults with ALL:

1. Complete MRD response: With an evaluation that meets a set of minimal technical standards for the method utilized, no MRD is discovered.
2. MRD Persistence: The existence of a continuously quantifiable MRD positive that can be measured at least two times with at least one significant treatment element in between.
3. Reappearance of MRD: Change from MRD negativity to measurable MRD positivity, ideally with emphasis from a second sample prior to a treatment change.

2.14 Clinical Importance of MRD in Childhood ALL

The MRD studies are an excellent approach to identify patients who require intensive therapy, which is more toxic, from patients who could be cured with less intensive therapy. Patients with MRD greater than 0.01, measurable MRD have a significant probability of relapse. This could help with one of the most difficult aspects of leukemia treatment. The MRD can identify leukemic cells that respond well to treatment right

after induction. The MRD is the most powerful predictor of outcome in ALL, and it is employed in therapy classification and regimens as a result.

Monitoring of MRD enables clinicians to detect ALL relapse risk, stratify chemotherapy intensity and duration, and determine the best time for hematopoietic stem cell transplantation in children with ALL.

2.15 Aim of MRD Studies for Clinical Purposes

1- The MRD level that should be considered while making a therapy decision.

2- The most instructive aspects in the therapeutic process.

3- The clinical utility of the information provided by each approach at various time points.

MRD is an essential predictor of post-transplant prognosis since it is such a significant predictor of therapeutic outcome.

2.16 The Flow Cytometry Technique

Moldavan described a photoelectric method for measuring cells in a capillary tube in 1934 (Moldavan, 1934). Fulwyler invented a cell sorter that used the Coulter principle to size cells and electrostatic charge of droplets to sort them in 1965, which marked the beginning of contemporary flow cytometry (Fulwyler, 1965). Paul Mullaney created multi-parameter flow cytometry (Campana & Coustan- Smith, 1999) after merging volume, light scatter, and fluorescence measurements into a single apparatus after a few years.

Gary Salzman's comprehensive experiments, on the other hand, added the capability of measuring side scatter. Fluorescence Activated Cell Sorter (FACS) was coined by

Leonard Herzenberg in the mid-1970s, when flow cytometers were first introduced to the market (Campana & Coustan- Smith, 1999).

In order to identify and sort cells within complicated populations, immunophenotyping employing flow cytometry has become the method of choice. Both basic research and clinical laboratories have used this technology. Immunophenotyping is effective in determining the cause and nature of leukemia, as well as the control of hematopoietic cell differentiation and maturation (Campana & Coustan- Smith, 1999).

The three major systems that make up a flow cytometer are fluidics, optics, and electronics. A flow cytometer's primary operation is to insert a tube holding the prepared cells under inquiry. The sample is taken from the sample vessel and pushed by tubing into the flow chamber (flow cell). Cells are shown to one or more light sources as they move through the flow chamber one at a time (Lasers). As cells pass through the flow chamber, the laser beam impacts them.

The physical features of each cell are revealed by the way light bounces off them. If fluorescent molecules are present on the particle, light scatter and or fluorescence are recorded, spectrally filtered, and sent to appropriate photo detectors for conversion to electrical signals, depending on the type of fluoro-chromes (Wohlfahrt et al., 2015).

Forward Scatter (FSC) and side scatter (SSC) are the two types of light scatter collected (SSC). Forward scatter is a technique that assesses the size of a cell by measuring scattered light in the direction of a laser line. The granularity of the cell is measured by measuring scattered light at a 90-degree angle to the laser stream.

The data is amplified and processed by the electronics in the cytometer. They transform analogue data to digital data, which is then saved in a computer. As shown in Figure 6, this information can be used to identify subpopulations within the sample (Dayan et al.,

2004).

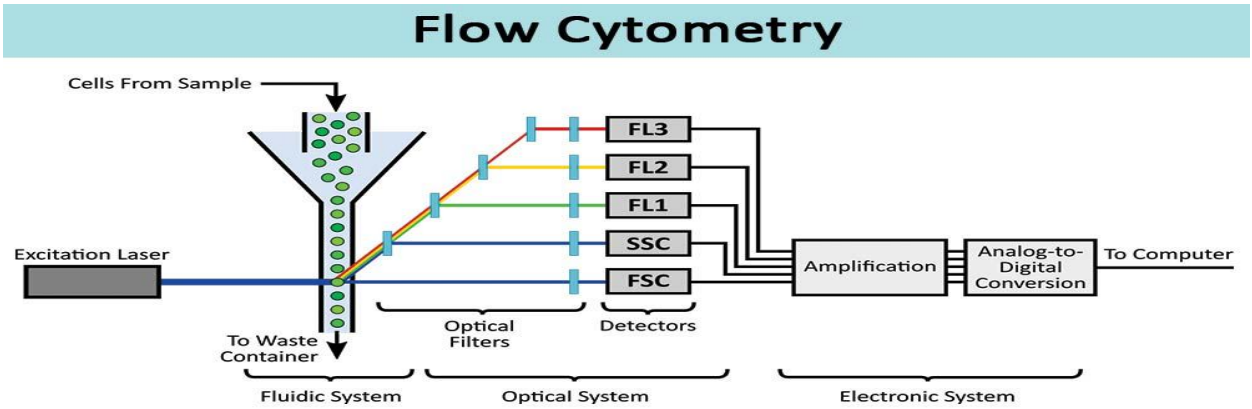

Figure 6: Light signals in flow cytometry (Dayan et al. 2004).

2.17 MRD in Palestine

One of the genuine success stories of modern medicine is pediatric ALL as indicated by (Othus et al., 2020).

Recognizing the Immunophenotyping of ALL in Palestinian children using 8-colour flow cytometry which was carried out at Beit Jala Hospital-Palestine/KHCC; the samples were obtained in Beit Jala Hospital and then sent to KHCC in Jordan. The test is conducted at zero time, at diagnoses, then at time point 2, which is on day 33, after the end of first dose of therapy.

Monitoring of MRD will be utilized to investigate the most effective method for regulating and evaluating the treatment plan from the time the disease is diagnosed, through therapy induction, and until the patient is in complete remission (Schuurhuis et al., 2018).

MRD is a term used to underline that when a patient is in remission, a tiny number of leukemic cells persists in the body, which is the most common reason of leukemia return (Pui et al., 2015). As a result, the researcher will emphasize the ideal use of MRD

in a therapeutic plan, as well as the importance of the time the test will be performed and the significance of immunoglobulin markers found through MRD measures in treatment decision making (Anderson et al., 2017).

MRD is the strongest sign in pediatric ALL, and one of the most critical components of treatment regimens and patient management through treatment or at the completion of induction remission therapy (Pui et al., 2015). In this chapter, what MRD is and how it can help to decide on a treatment plan for child with ALL will be described. What immunoglobulin markers MRD can provide, how important they are in patient stratification, and if there are any links between these immunoglobulin markers and treatment response or relapses will all be described as well (dxalal, 2019).

Chapter Three:

Conceptual Framework

3.1 Conceptual Framework

This chapter discusses the variables that influence the conceptual framework components for the Immunophenotyping and MRD in Palestinian children less than 15 years old with ALL.

Figure 7 depicts the study's conceptual framework, which includes the following variables:

- Patient's demographics (patient's age, gender, place of birth.)
- Laboratory findings (white blood cell, hemoglobin, platelets, lactate dehydrogenase, MRD, B- Cell, T-cell, CD10, CD19(D), CD34, CD20(P), HLA-DR, cCD79a, CD58, nTdT, TCRδ/γ, CD2, cCD3, sCD3, CD5(P,70%), CD7, CD38(P), CD4 and CD8).

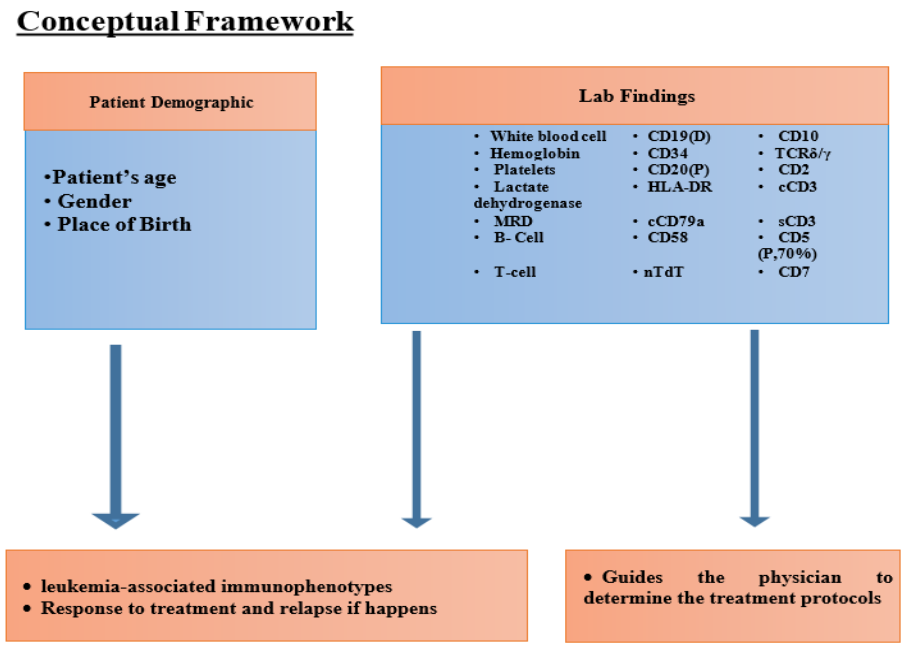

Figure 7**:** Conceptual Framework of the Study

3.2 Operational Framework

Operational definitions must be determined before the collection of data starts. Assigned staff that is responsible to access the system must have identical understanding, knowledge, and skills for retrieving data using the same steps. Collecting data is a systematic and consistent procedure that requires well-trained staff to perform the tasks effectively, the same protocol is constructed to be applied by all team members in the same manner; this will help confirm validity and reliability of data, and it was conducted through a comprehensive operational definition of terms.

The conceptual framework includes several independent factors associated with the structured teaching program of Acute Lymphoblastic Leukaemia, which is divided into patient demographics, laboratory findings and other risk factors. The mentioned factors were utilized to create the conceptual framework of the current study. The current chapter is considered as the foundation for the result analysis in addition to discussion and conclusion.

Chapter Four:

Methodology

4.1 Introduction

This study used a descriptive design approach retrospective study to attain the goals of the research. The data collection was based on two data sources: medical information about patients with ALL that received treatment in Beit Jala Hospital, and records of targeted patients under this research, including MRD findings received from KHCC in Jordan.

There is a debate about the best timing to do MRD test; different previous studies showed that the day-28 MRD test was prognostic, while other studies reported that the test at day 15, day 29, or day 78 according to the protocol of treatment adopted(Pulsipher et al., 2015). In this study, we adopted the MRD test at the day 33 (the adopter treatment protocol in the hospital) as directed by Doctor Mohammad Najajrah in charge of treating Palestinian children with ALL at Beit Jala Hospital.

4.2 Study Area/ Setting

This research was conducted in Beit Jala Hospital, West Bank (WB), Palestine. The total hospital capacity was about 135 beds, including pediatric and adult oncology, kidney, surgery, internal medicine, delivery, emergency, and ICU departments. The data collectors in the hospital were approached and reviewed by researcher.

Study population

The sample of the study (medical records) is 84 patients.

4.3 Study Subjects

Criteria for Inclusion

1. All patients treated with (ALL) patients in Beit Jala Governmental Hospital and have a follow up in King Hussein Cancer Center in Jordan.
2. (ALL) Children patients under 15 years old.
3. Patients must be diagnosed specific for (ALL), and treated with chemotherapy.
4. All medical records for (ALL) patients from 2018 to 2021.

Criteria for Exclusion

All incomplete medical records of the above-mentioned inclusion were excluded from the study.

4.4 Study Design Overview

This study employs a retrospective study in quantitative descriptive research direction. This study proceeded using the medical records of ALL patients treated at Beit Jala Hospital as a source of data. These patients' records include all necessary information regarding the study's participants, including MRD results from KHCC. The findings will be evaluated to determine which immunoglobulin markers are most prevalent among the patients under investigation. White blood cell count (WBC), hemoglobin (Hb), and platelet count will be collected from the electronic medical record (EMR) that is used in government hospitals, including Beit Jala Hospital (Plt).

4.4.1 Dataset Reported Cases Analysis

The dataset of reported cases, patients who are diagnosed and treated in this hospital with complete medical record, was examined by analyzing the data in the electronic

medical record (EMR) related to ministry of health called Avicenna and paper based records. Missing data for variables collected in medical records and data processing activities such as comparability, completeness, and validity are used to calculate the overall efficacy of the data.

4.4.2 Data Collection

Data collection was conducted for review included medical reports between March 2018 and June 2021 at the Beit Jala Hospital in the West Bank, and this was done by researcher.

The dataset extracted from paper and electronic medical records the follow the goals of the research and its conceptual framework was classified into three categories; the first is patient's demographics, which identifies the patient's age, gender, diagnoses, and date of diagnoses. And the second category is related to laboratory findings, which are white blood cells, hemoglobin, platelets, and lactate dehydrogenase. The results are assessed based on the normal ranges which are accredited by MOH, MRD, B Cell, T Cell, CD10, CD19(D), CD34, CD20(P), HLA-DR, cCD79a, CD58, nTdT, TCRδ/γ, CD2, cCD3, sCD3, CD5(P,70%), CD7, CD38(P), CD4 and CD8. All of these results were written on the report by mentioning the positive markers as existed, and no mentioning for negative.

4.5 Data Management & Analysis

In this research, the records were evaluated by the researcher, and from the original cancer report, a summary of each case report was created. We summarized the patient output impact using descriptive statistics, such as frequencies and percentages for

variables, in accordance with our research objectives. The statistical analysis employed the confidence interval (CI), P-value, and prevalence. IBM SPSS 22 was used to enter data, clean it up, and analyze it (IBM Corporation, 2016). To investigate the relationship between outcome availability and independent variables, a multi-variate regression analysis model was put up.

4.6 Ethical Consideration

As this study uses a secondary data, informed consent form is not required. No identifiers or personal information, such as participants' names, IDs, or other information used were collected or retained, ensuring complete privacy and confidentiality. The Palestinian Ministry of Health gave its clearance to utilize Avicenna as the formal system for medical information; data was gathered with the Ministry's permission. Graduate studies proposal permission was acquired by the Arab American University of Palestine (AAUP).

Chapter Five:

Results

This chapter includes actual results and findings of the research. The chapter includes charts, tables, and graphs as well as a story that describes what is considered the most applicable information. Also, it includes an explanation and comparison of our research results and other researchers' studies.

This study aimed to assess and study MRD in Palestinian children patients' suffering from ALL and find its impact on the treatment protocol.

The research focused on ALL patients who were less than 15 years old treated in Beit Jala Governmental Hospital located in the south area of Palestine. The researcher depended on either data collected from patients' files papers or electronic extracted from electronic medical records stored in a health information system known as Avicenna operated in governmental hospitals including Beit Jala from 2018 until mid of 2021.

The researcher collected the results of flow cytometry for Palestinian children with ALL that were conducted in King Hussein Cancer Centre in Jordan, since it is not conducted nor carried out in Beit Jala or any other governmental hospital.

Also, this chapter describes the characteristics of the targeted group including demographic data as well as a summary of the study findings.

The study included demographic variables such as age, sex, place of birth, diagnosis, and date of diagnosis, in addition to laboratory results including white blood cell, haemoglobin, platelets, lactate dehydrogenase, MRD, B Cell, T Cell, CD10, CD19(D), CD34, CD20(P),HLA-DR, cCD79a, CD58, nTdT, TCRδ/γ, CD2, cCD3, sCD3, CD5(P,70%), CD7, CD38(P), CD4 and CD8.

Missing data was excluded from the study variables; all identification variables were excluded to protect the privacy of patients. The overall cases remained after excluding the missing data was 84 cases.

5.1 Descriptive Statistics

Table 5: the different percentages of variables under study

Variable	Value	Frequency	Percentage	Valid	Missing
Age groups	1-4	38	45.3	84	0
	5-7	28	33.3		
	8-10	8	9.5		
	11-15	10	11.9		
Place of birth	Bethlehem	14	16.7	84	0
	Gaza	32	38.1		
	Hebron	32	38.1		
	Nablus	1	1.2		
	Jerusalem	1	1.2		
	Ramallah	3	3.5		
	Tulkarem	1	1.2		
Gender	Male	44	52.4	84	0
	Female	40	47.6		
Diagnosis	B-Cell ALL	46	54.8	84	0
	Pre B-Cell ALL	23	27.4		
	T-Cell ALL	14	16.6		
	B-Cell All down synd.	1	1.2		
MRD at 33 day	Negative	66	78.6	84	0
	Positive	18	21.4		
B Cell	Negative	37	44	84	0
	Positive	47	56		
Precursor B Cell	Negative	61	72.6	84	0
	Positive	23	27.4		
T Cell	Negative	70	83.3	84	0

	Positive	14	16.7		
CD10 CD19	Negative Positive	39 45	46.4 53.6	84	0
CD19 (D) HLA-DR, sCD3	Negative Positive	81 3	96.4 3.6	84	0
CD34	Negative Positive	62 22	73.8 26.2	84	0
CD20 (P)	Negative Positive	60 24	71.4 28.6	84	0
cCD79a	Negative Positive	83 1	97.6 2.4	83	1
CD58	Negative Positive	47 37	56 44	84	0
nTdT CD2	Negative Positive	82 2	97.6 2.4	84	0
TCRδ/γ	Negative Positive	84	100	84	0
sCD32 CD38 (P)	Negative Positive	83 1	98.8 1.2	84	0
CD7 CD20 (D)	Negative Positive	79 5	94 6	84	0
CD38	Negative Positive	57 27	67.9 32.1	84	0
CD34(p)	Negative Positive	65 19	77.4 22.6	84	0

ALL children patients among males and females: 44 males with a percentage of 52.4 and 40 females with a percentage of 47.6%.

Age under study covers all children ages from1 year old to 13 years old classified in four age groups (1-4, 5-7, 8-10, and 11-15), same done by (Buckley et al., 1989), with the highest percentage in the first age group (1-4) 45.2 followed by the second age group (5-7) with a percentage of 33.3. The third age group (8-10) has 9.5 percentages and the last age group (11-15) has 11.9 percentages as shown in figure 8.

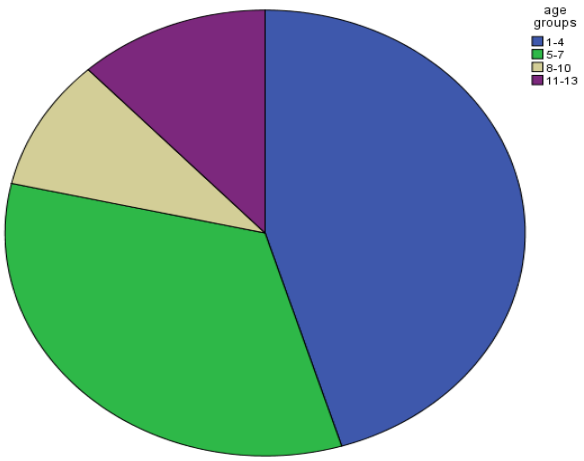

Figure 8: Age group distribution

Regarding place of birth, the highest percentages were in Gaza and Hebron with 38.1% each (most patients treated in this were hospital from these two governances) followed by Bethlehem 16.7%, patients from cities in north Palestine are treated in hospitals like Al Najah hospital, as shown in figure 9.

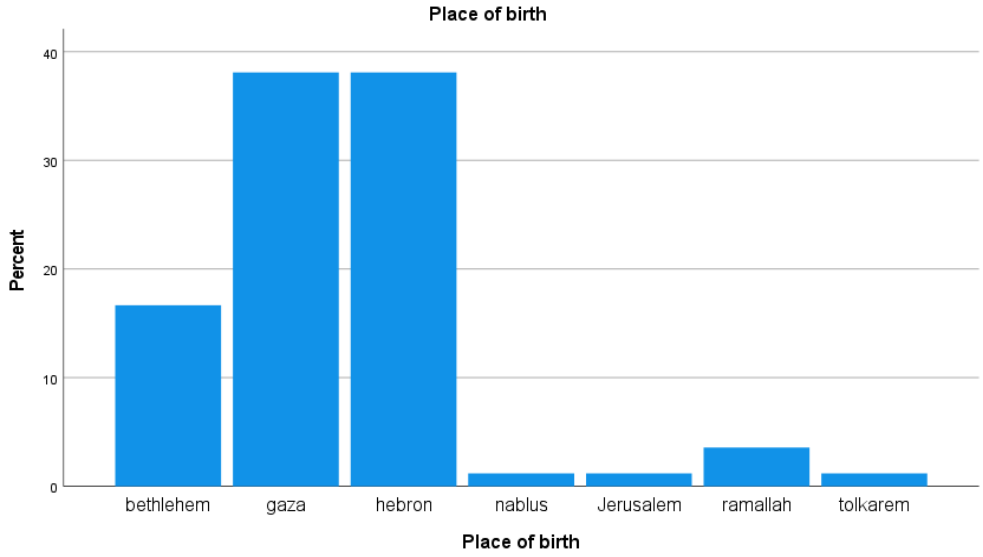

Figure 9: ALL children distributed by place of birth

For diagnoses, variables the percentages were arrange in descending as B cell, precursor B cell, T cell, and B cell ALL Down syndrome, with rates of 54.8%, 27.3%, 16.6%, and 1.2% respectively as illustrated in figure 10.

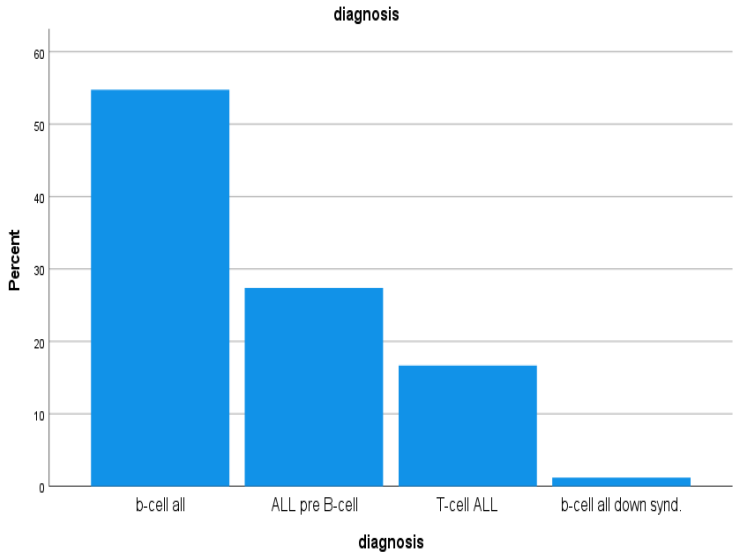

Figure 10: ALL children patients among diagnoses variable under study

The MRD at 33 days was recorded negative in 66 cases with a percentage of 78.6 and positive in 18 cases (9 males and 9 females) with a percentage of 21.4 as appears in figure 11.

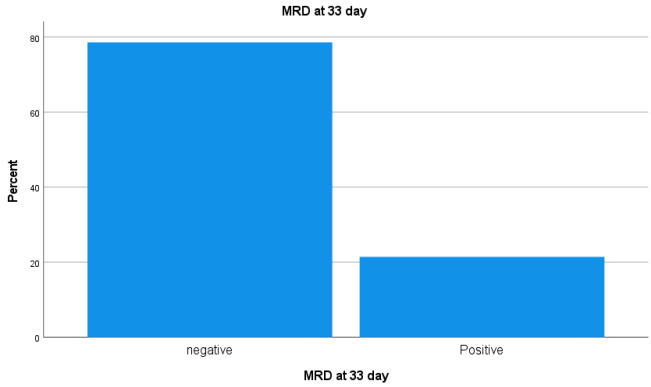

Figure 11: ALL children patients among MRD at day 33 variable under study

The B – Cell was recorded negative in 37 cases with 44% and positive in 47 cases with 56% (including Down syndrome). Whereas precursor B – Cell reported as negative in 61 cases with 72.6% and positive in 23 cases with 27.4%.

The T – Cell was found to be negative in 70 cases with 83.3%, and positive in 14 cases with 16.6%. Regarding the CD markers, the results were found to be as follows:

The CD10 and CD19 recorded in 39 cases as negative and in 45 cases as positive with percentages 46.4 and 53.6 respectively.

The CD19 (D), HLA-DR, sCD3, CD38 (D), CD34 (D) and CD58 (D) were negative in 81 cases with 96.4% and positive in 3 cases with 3.6%. The CD34 reported to be negative in 62 cases with 73.8% and positive in 22 cases with 26.2%. TheCD20 (P) was negative in 60 cases with 71.4% and positive in 24 cases with 28.6%.

The cCD79a was found to be negative in 81 cases with 96.4% and positive in 2 cases with 2.4% and 1 case reported as a system missing value. The CD58 reported negative in 47 cases with 56% and positive in 37 cases with 44%. The nTdT, CD2, CD5 (P, 70%), CD20, and CD33 were confirmed as negative in 82 cases with 97.6% and positive in 2 cases with 2.4%.

The TCRδ/γ was reported as negative in all cases. The CD7 and CD20 (D) were reported as negative in 79 cases with 94% and positive in 5 cases with 6%. The sCD32, CD38 (P), CD4, CD8, CD2 (D), CD1a (P), nTdT (D), CD10 (B), CD5, CD1a, and CD33 (D), were found to be negative in 83 cases with 98.8% and positive in 1 case with 1.2%.

The CD38 was found to benegative in 57 cases with 67.9% and positive in 27 cases with 32.1%, while the CD34 (P) was found to benegative in 65 cases with 77.4% and positive in 19 cases with 22.6%.

According to the finding of this study, we found that there was no association between MRD at day 33 and gender (p value = 0.820), and between MRD at day 33 and age groups (p value= 0.715). The results also showed that there was no association between MRD at the day 33 and the WBC. There was also no association between platelets and LDH (p value > 0.05) but, there was an association with the HB (p value = 0.008).

Table 6: Relation between MRD at day 33 and WBC

	MRD at 33 day	N	Mean	Std. Deviation	Std. Error Mean	T-test/ p value
WBC	Positive	18	9.1278	7.05189	1.66215	0.317
	negative	66	26.6561	73.46136	9.04247	

Table 7: Relation between MRD at day 33 and PLTs

	MRD at 33 day	N	Mean	Std. Deviation	Std. Error Mean	T-test/ p value
PLT	Positive	18	96.67	87.209	20.555	
	negative	66	84.95	93.212	11.474	0.633

Table 8: Relation between MRD at day 33 and LDH

	MRD at 33 day	N	Mean	Std. Deviation	Std. Error Mean	T-test/ p value
LDH	Positive	18	1206.61	3534.355	833.056	
	negative	66	999.32	1235.744	152.110	0.690

Table 9: Relation between MRD at day 33 and HB

	MRD at 33 day	N	Mean	Std. Deviation	Std. Error Mean
HB	Positive	18	10.1667	1.42127	0.33500
	negative	66	9.0530	1.57414	0.19376

Table 10: Binary logistic regression between MRD at day 33 and HB

	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**
HB Step1a	0.475	0.188	6.398	1.0	0.011	1.608
Constant	-5.863	1.879	9.741	1.0	0.002	0.003

a. Variable(s) entered on step 1: HB.

As cleared from the table above the coefficient of HB was 0.475, which means that the regression equation $p(x) = e^{y}/ (1+e^{y})$ where Y=-5.863+0.475HB, here HB considered a predictor variable whereas Y represents the dependent variable MRD at day 33, where one represents the existence of MRD at day 33 in the patient's blood and zero means the absence of MRD at day 33from patient's blood.

The HB is statistically significant at level of 0.05 significance, which means that any changes in the HB variable are associated with changes in the probability of the absence

of MRD at day 33 in the patient's blood, since the coefficient of HB variable is greater than zero, (0.475), which it means that the higher changes in the absence of MRD at day 33 is associated with the higher changes in HB variable.

The EXP $^{(B)}$ in the table above =1.608, means that the probability of absence of MRD at day 33 increases 1.608 times for every unit increase in the count of HB.

We recommend the creation of a national electronic registry for ALL children patients to better track all cases both in the government as well as the non-government hospitals. Coordination between academic institutions and Palestinian Ministry of Health regarding the research topics of post graduate students in the field of health to close gaps would be of great value.

Our findings also showed that there was no association between MRD at day 33 and ALL B cell, ALL precursors B cell, and ALL T cell (p value > 0.05). Also, we found that there was no association between LDH and ALL B cell, ALL precursors B cell, and T cell (p value > 0.05).

According to our findings there was no association between B- cell, p B-cell, and T- cell (P value> 0.05) with platelets. There was also no association between B- cell, P B- cell and T-cell with LDH (P value >0.05). In addition, there was no association between B-cell, P B-cell, T- cell and WBC.

Our results showed no association between diagnosis and MRD at day 33, (P value >0.05).

Chapter Six:

Discussion and Conclusion

The main findings regarding the research questions are abstracted, and conclusions are described depending on the findings of the studies presented in this thesis. In addition, the strengths and limitations of this thesis are considered, and recommendations are given for further research. In this chapter we also provided policy makers and researchers with suitable recommendations.

Since Leukemia is a life-threatening disease that leads to the death of many people worldwide, looking for the best way to treat patients at the early stages would save lives for both adults and children. This study shed light on the most common cancer for pediatric which is CALL as stated by (Greaves, 2018)(Bachir et al., 2009).

To our best knowledge, it is the first research work to be conducted in Palestine about children with ALL to find out if the globally adopted protocols that link MRD with the treatment's paths can be applied on them. According to Jovanovska, the presence of MRD at the end of induction therapy (day 33) is associated with poor blast clearance (Jovanovska et al., 2019). Patients with poor response to early treatment and detectable MRD had worse prognosis and had high risk of relapse (Anderson et al., 2017). According to previous studies, there are many methods and factors affecting the way ALL is assessed and treated; one of these methods is the MRD, which describes the small number of cancer cells found in the patients' body after treatment. If the MRD is positive it indicates, the disease is still present; however, if it is negative, it is an indication that the disease is absent.

The MRD is a very important test since the remaining cancer cells might be very small to be detected by the normal test such as scanning under the microscope.

Physicians use MRD as an indicator to scale the treatment effectiveness and a way to find cases or patients at risk of relapse. In addition, it aids them to monitor and confirm remissions, and probably to conclude the relapse of the disease at early stages. The existence of MRD after treatment means not all of the cancer-affected cells responded to the cure or the cancer-affected cells became medication resistant. Different studies indicated a strong link between MRD and the risk for relapse. The use of MRD in diagnosis and treatment protocol was applied in Beit Jala Hospital like global protocols, but as mentioned by Dr. Najajra there is no follow up protocols after treatment for cured patients.

Recently,(Greaves, 2018)(Melton, 2019) "*A Panel of Experts in Adult and Paediatric Leukaemia*" assured that ALL currently is the first hematologic neoplasm for which assessment of early response to treatment by MRD test has shown to be a significant technique for guiding ALL patients' therapy.

In this study, Palestinian children under 15 years old who had ALL were examined and the existence of MRD in their bodies after treatment was studied using the results found in their files by applying 8–color flow cytometry method.

There are three types of leukemia that could be found in patients suffering from ALL: B-cell, precursor B-cell and T-cell which are identified using MRD test.

The presence of 0.01 percent or more ALL cells indicates that the MRD is positive (Campana, 2009), while (Sas et al., 2019) stated that MRD as a time point 1 was used to stratify patients into low, middle, and high risk groups on the day 33 of treatment.

According to our findings, the MRD at day 33 was positive in 10.7% in patients who

had ALL B-cell, 6% in patients with ALL precursors B-cell and 3.6 % in ALL T-cell patients. As MRD positive here means these patients needed intensive therapy or may need HSCT to reach remission. The percentage of male and female patients who had MRD at day 33 was the same (10.7%).

The Complete Blood Count (CBC) test is used to determine the count of white blood cells and platelets, in addition to the amount of hemoglobin in the red blood cells in order to diagnose leukemia. Blood components are used as indicator for early diagnosis, any abnormality that appears needs more tests to confirm the presence of the disease.

This study reveals that ALL B-cell appeared in 54.8% of the patients understudy, whereas (Shawahna et al., 2021)in a study conducted in Palestine found that the B cell is present in 79.7% of patients. ALL T-cell appeared in 16.7% of patients, which is close to the result shown by (Shawahna et al., 2021), (20.3%). According to (Teachey & Pui, 2019), the precursor B cell ALL existed in 80% of cases, whereas in our results precursor ALL B cell constitutes 27.4%, about one third of the result reported by (Teachey & Pui, 2019). Mature B cell ALL were shown in about 2-5% of cases, our results showed that it is a 54.8%, T cell ALL existed in about 15-17% of cases which complies with our results (16.7%).

We found that a precursor B cell is linked with better prognosis and this is a similar result obtained by (Teachey & Pui, 2019; Von Stumm & Plomin, 2015)

Findings in this study regarding T-cell is close to the result found in (Wimalachandra et al., 2020) in their research that was conducted in Sri Lanka between 2009 and 2013 regarding 229 patients with 67% children less than 12 years old (20%) but far from their findings regarding to B-cell (80%); their sample of study was bigger than this study's but had the same results found by (Wimalachandra et al., 2020).

It seems that there is no clear pattern regarding the types of leukemia at diagnosis of children patients with ALL.

Different markers appear in various types of leukaemia; some specific for ALL B-cells, others exist in ALL T-cells, and some appear in both (all types).

Cluster of Differentiation (CD) markers are a good tool to identify different types of leukemia and diagnose ALL. As appeared in this study, markers such as CD10, CD34, CD20 (P), CD38, CD58, and CD19 play a primary role from both diagnostic and prognosis point of view that are linked to pediatric ALL cancer; therefore, leading to better treatment protocols and more lives saved.

According to (Boer et al., 2016), one of the factors to consider a case precursor B cell ALL is if the cells expressed surface CD19 and CD10. Our findings showed that CD10 existed in 53.6% of patients under study, 37% in ALL B-cell and 16.6% in ALL precursors B cell. It was found by (Chiaretti et al., 2014) that it is nearly always co-express in ALL B-cell (nearly 54%). This result is in accordance with our results. The expression of CD10 is also quite common (25%) in T-cell as indicated by (Jalal et al., 2017).

According to (Wimalachandra et al., 2020), 93% percent of children with ALL B-cell tested positive for CD10, which is not coincident with our study (37%). A study by (Berhili et al., 2021) on a 8 year-old boy with ALL B-cell from Morocco in the year 2021, found that the percentage of CD10 was 97%; both studies recorded a very high existence of CD10 marker for B-cell in paediatric ALL patients, which can be considered as a specific marker for ALL B-cell in children.

The CD34 existed in about quarter of patients under research (26%), 16.5% in B-cell and 9.5% in precursor B-cell respectively. It is considered as one of precursor B-cell

and also a mature B-cell identifiers according to (Boer et al., 2016). It is also considered a nearly always co-express with both ALL B-cell patients as reported by (Chiaretti et al., 2014), and a co-express in ALL T-cell as indicated by (Cannizzo et al., 2011). It is also considered by (Jalal et al., 2017) as a precursor T-cell ALL which contradicts to our findings regarding the existence of this marker in T-cell (1%).

In a study by (Wimalachandra et al., 2020), the percentage of CD34 in children was 69.5 in ALL B-cell, while ours was 26.2% in T-cell, whereas (Berhili et al., 2021) found it to be 90%. Therefore, the CD34 can be considered as a marker for ALL B-cell children patients.

The CD20 (P) was found in 29% of examined patients' files. 23% was present in ALL B-cell and 6% in ALL precursors B-cell, and was not shown in any patients with ALL T-cell; it was one of precursor B-cell as reported by (Boer et al., 2016). According to (Pavlasova & Mraz, 2020), the CD20 can be classified as a general marker pertained to ALL B-cell.

Another study conducted in India, found that the percentage of CD20 was 62 % in ALL precursor B-cell patients on a study sample of hundred patients aged (4 months to 65) who had ALL precursor B-cell (Kumar et al., 2014).

The CD58 was found in 44% of patients: 27.4% in ALL B-cells and 13% in ALL precursor B-cells, whereas 2.4% in ALL T-cell. A study conducted in 2017 in India found that about MRD analysis using flow cytometry in precursor B-cell for 73 patients aged 1 to 40. The study found that the marker CD58 was in 43 patients with 58.9 percentage (Jain et al., 2018).

The CD38 appeared in 32% of patients; 24% in B-cells and 8% in precursor B-cell; whereas in (Jain et al., 2018), it was under expressed in 60 patients with 82.19

percentage. According to (Tembhare et al., 2020), the CD38 tested was positive in 79.1% in patients with T-cell; but in this study, this marker did not appear in patients with T-cell, as mentioned before there is no cleared or confirmed pattern in leukaemia.

The CD19 appeared in 54% of patients in our study; 37% in B-cell and 15.5% in precursor B-cell; it was considered to be nearly always co-express with ALL B-cell (highest percentage in our study) as shown by (Chiaretti et al., 2014). It was found in a hundred percent with ALL B-cell children patients according to the study conducted by (Wimalachandra et al., 2020),which is nearly double the percentage of our results. Also, the percentage was 97% in the case presented by (Berhili et al., 2021), so it is a good marker for B-cell paediatric patients.

The CD7 was found positive in 6% of patients in our research with ALL T-cell and zero percent in B-cell, with the CD7 nearly always co-express in T-cell ALL according to (Cannizzo et al., 2011).It is a very low percentage, compared with results found by (Wimalachandra et al., 2020).68.8%) found in children patients with T-cell. Whereas (Jalal et al., 2017) classified CD7 as a precursor T-cell ALL. The CD7 is considered as a marker linked to patients with ALL T-cell(Wimalachandra et al., 2020).

It seems that there is no specific pattern regarding the appearance of markers in ALL patients. Some markers are over expressed whereas others are under expressed, some which appear more in patients with B-cell or precursor B-cell, whereas others show more or less with T-cell. As shown from previous studies, there was no standard patterns regarding the existence of markers in patients suffering from ALL and it seems that there are no significant results between children and adults.

Patients with T-cell ALL tend to be older than those with precursor B-cell ALL as reported by (Tomizawa & Kiyokawa, 2017). By using chi square; Person Correlation

with p value< 0.001, lambda= 0.601, gamma= 0.017 and an association between age groups and B-cell; with p value <0.011, lambda= 1.52, gamma= 0.019 but no association between age group with precursor B-cell, gamma= 0.796; Our findings found an association between age groups and T-cell (the older the patient is, the more likely to be a T-cell patient).

Unfavorable variables are more common in patients with T-cell ALL, such as male, age greater than nine years, and high WBC count at diagnosis as reported by (Wahhab, 2015). Our results showed that 11 males and 3 females had T-cell, whereas there was no association with white blood cells (p value >0.05).

According to a study by (Zahra et al., 2021) on 75 patients with mean age of 4.7 years, there was a link between LDH levels on the first day of treatment and each age group. However, there was no association with gender. Our finding showed an association between LDH and age groups (p value = 0.004) and no association with gender.

In a study by (Murali et al., 2017),there was an association between LDH and ALL patients, whereas our finding showed no association with any of leukemia patients (p value > 0.05). In a study by (Elbossaty, 2017), there was an association between LDH level and ALL patients and also there was an association with WBC, which did not match with our findings, indicating no association either with leukemia or WBC count.

To conclude, MRD is a reliable test that can serve ALL children patients, and can play a primary role in predicting the disease. MRD should be done not only at day 33 after treatment, but also all through the follow up of patients.

Although the survival rate is high (80%) according to previous studies, but there is still a relapse in 20% of ALL patients with a cure rate of (25% - 40%). Any life lost counts and focus on ALL children patients should be at the highest concern at the national

level.

SPSS program was used and the independent T- sample test was done and a significant difference between means of MRD at day 33 and WBC was found as shown in table 6.

The same test showed that there was a significant difference in means between MRD at day 33 with PLTs, and LDH as clear from table7 and table 8.

That's why these above variables where excluded from the logistic regression. However, there was no significant difference between the means of MRD at day 33 and HB, so the HB variable was included in the logistic regression as shown in table 9.

A binary logistic regression was applied after that to the variable of MRD at the day 33 and HB as shown in table10.

The researcher advises decision makers in The Ministry of Health to create a specialized central lab for Leukemia patients and to foster relations regarding Leukemia with neighboring countries to exchange expertise and conduct scientific research, as well as to encourage the government to facilitate the participation of leukemia specialists' physicians in international conferences and workshops pertained to disease.

There must be a collaboration between this hospital and the labs that do the MRD test instead of sending samples outside the country.

Finally, we encourage other researchers to conduct future studies about Palestinian ALL adult patients and to see the effect of MRD in predicting the disease to better track and therefore, better treat the disease, because discovering the disease at early stages has a positive influence in the treatment process.

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APPENDICES

Appendix I A letter of facilitating a research mission from the Arab American University

Arab American University
Faculty of Graduate Studies

الجامعة العربية الأمريكية
كلية الدراسات العليا

2021/6/2

الى من يهمه الامر

تسهيل مهمة بحثية

تحية طيبة وبعد،

تهديكم الجامعة العربية الامريكية أطيب التحيات، وبالإشارة الى الموضوع أعلاه، تشهد كلية الدراسات العليا في الجامعة أن الطالبة نسرين سليم احمد نزال، والتي تحمل الرقم الجامعي 201820295 هي طالبة ماجستير في الجامعة العربية الامريكية تخصص معلوماتية صحية وتعمل على اعداد رسالة الماجستير لاستكمال متطلبات الدرجة، نأمل من حضرتكم الايعاز لمن يلزم لمساعدتها للحصول على المعلومات اللازمة، علماً ان هذه المعلومات ستستخدم لغاية البحث فقط وسيتم التعامل معها بغاية السرية.وقد أعطيت هذه الرسالة بناءً على طلبها.

شاكرين لكم تعاونكم

كلية الراسات العليا

كلية الدراسات العليا
FACULTY OF GRADUATE STUDIES

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الملخص

التنميط المناعي والأمراض المتبقية في الأطفال الفلسطينيين دون سن 15 عامًا المصابين بسرطان الدم الليمفاوي الحاد: دراسة بأثر رجعي من مستشفى بيت جالا الحكومي

سلطت هذه الدراسة الضوء على سرطان الدم الذي يحدث نتيجة اضطراب العديد من خلايا الدم بسبب خلل في نخاع العظام (Hunger & Mullighan, 2015) وهو يؤثر في الغالب على كبار السن الذين تتراوح أعمارهم بين 55 سنة وأكثر ولكنه أيضا سرطان معروف لدى الأطفال الذين تقل أعمارهم عن 15 سنة (Atkin et al., 2017). ركزت هذه الدراسة الوصفية بأثر رجعي والتي تعتمد النهج الكمي على سرطان شائع وهو سرطان الدم الليمفاوي الحاد في مرحلة الطفولة. واستهدفت 84 مريضا؛ 44 ذكرا و40 أنثى مقسمين إلى أربع فئات عمرية (1-4 و5-7 و8-10 و11-13). تم جمع بيانات المرضى من السجلات الطبية المخزنة في مستشفى بيت جالا في الضفة الغربية / فلسطين. والنتائج التي تم الحصول عليها من مركز الحسين للسرطان في الأردن.

الهدف الرئيسي من هذا البحث هو اختبار Minimal Residual Disease في اليوم 33 خلال العلاج وهو الاختبار الذي يكشف عن وجود خلايا اللوكيميا (B-cells، T-cells، Precursor B-cells) التي لا تزال في دم المريض وتصنف كسبب رئيسي لرجوع السرطان مرة أخرى (Testing & Cancers, 2020) ويمكن أن تلعب دورا هاما في تحديد بروتوكول العلاج. كانت الطريقة المستخدمة في هذا البحث للكشف عن Minimal Residual Disease هي flow cytometry بثمانية ألوان. حددت هذه الدراسة immunoglobulin markers المهيمنة بين المرضى المستهدفين وفحصت عدد خلايا الدم البيضاء والهيموغلوبين وعدد الصفائح الدموية.

وأظهرت نتائج هذه الدراسة أن نتيجة فحص Minimal Residual Diseaseجاءت إيجابية في 18 مريضا (9 ذكور و 9 إناث) بنسبة 21.4٪؛ 10.7 في المائة من الذكور و 10.7 في المائة من الإناث. كذلك كانت إيجابية 10.7٪ فيالمرضى الذين لديهم B-cells، و 6٪ في المرضى الذين لديهم precursor B-cells، و 3.6٪ في المرضى الذين لديهم T-cells.

نسبة المرضى الذين لديهم B-cells كانت ٪54.8، والذين لديهم precursor B-cells كانت 27.4٪، بينما المرضى الذين لديهم T-cells كانت 16.7%. كما ظهر في هذه الدراسة فإن CD10، CD34، CD20 (P)، CD38، CD58، وCD19 تعتبر من ال markers التي يمكن

أن تلعب دورا رئيسيا في التشخيص والتكهن في حدوث المرض على حد سواء والمرتبطة بسرطان الأطفال، وبالتالي تكون بروتوكولات العلاج أفضل وتؤدي إلى إنقاذ المزيد من الأرواح.
اما بالنسبة الى CD10فقد تبين انها موجودة في 53.6٪ من المرضى قيد الدراسة: 35.7٪ من المرضى الذين لديهم B-cells و 16.6٪ من المرضى الذين لديهم precursor B-cells.
وظهرت CD34 في حوالي ربع المرضى قيد البحث (26٪): 15.5٪ من المرضى الذين لديهم B-cells و 9.5٪ من المرضى الذين لديهم precursor B-cells. اما(P) CD20فقد ظهر في 29٪ من ملفات المرضى التي تم فحصها من قبل الباحث، 23٪ من المرضى الذين لديهم B-cells و 6٪ من المرضى الذين لديهم precursor B-cells. CD58ظهرفي 44٪ من المرضى. 27.4٪ ممن لديهم B-cells و 13٪ ممن لديهم precursor B-cells في حين كانت نسبته 2.4٪ في المرضى الذين لديهم T-cells.
أيضا بالنسبة الىCD38فقد ظهر في 32٪ من المرضى; 24٪ ممن لديهم B-cells و8٪ ممن لديهم precursor B-cells. بينما CD19 ظهر في 54٪ من المرضى قيد الدراسة. 37٪ ممن لديهم B-cells و15.5٪ من المرضى الذين لديهم خلايا precursor B-cells. CD7 ظهر في 6٪ من المرضى الذين يعانون من T-cells وصفر في المئة في المرضى الذين لديهم خلايا B-cells. كما هو واضح من النتائج التي توصلنا إليها ارتبطت معظم العلامات (markers)إما مع المرضى الذين ظهرت لديهم خلايا B-cells أو خلايا precursor B-cells بينما CD7 يمكن أن ينظر إليه كعلامة(marker) موجود في المرضى الذين لديهم T-cells على الرغم من أن نسبته كانت منخفضة.