Brief Overview about Acute Lymphoblastic Leukemia and Its Molecular subtypes

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Abstract

Background: Acute lymphocytic leukemia (ALL) is a malignancy of B or T lymphoblasts characterized by uncontrolled proliferation of abnormal, immature lymphocytes and their progenitors which ultimately leads to the replacement of bone marrow elements and other lymphoid organs resulting in a characteristic disease pattern. ALL accounts for approximately 2 percent of the lymphoid neoplasms in the United States and occurs slightly more frequently in males than females, and three times as frequently in Caucasians as in African-Americans. Patients typically present with symptoms related to anemia, thrombocytopenia, and neutropenia due to the replacement of the bone marrow with the tumor. Symptoms can include fatigue, easy or spontaneous bruising and/or bleeding, and infections. About 75% of childhood ALL patients have recurring chromosomal alterations either aneuploidy or translocations, which can be detected by methods varying from karyotyping which identifies large alterations to whole genome sequencing which detect cryptic changes in the entire genome. Identification of these abnormalities is crucial for optimum disease evaluation, risk stratification, and treatment planning. Up to 10% of childhood ALL is T-ALL however, B-ALL represents 90%, including 31% with B-ALL with high hyperdiploidy, 21% B-ALL with ETV6::RUNX1, 7% other cytogenetic abnormalities (e.g.: B-ALL with BCR::ABL1, ALL with KMT2A, nearhaploidy/low haploidy, B-ALL with TCF3::PBX1, B-ALL with TCF3::HLF and B-ALL with IGH::IL3), and 30% of B-ALL with not identified specific genetic abnormalities, termed "B-other"

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Introduction

Acute lymphoblastic leukemia (ALL) is a group of malignant hematological neoplasms, characterized by abnormal proliferation of immature lymphoid progenitors, which accumulate in bone marrow replacing the normal hematopoietic cells, or in peripheral blood, and other extramedullary sites causing tissue damage. World Health Organization (WHO) classifies ALL into two major categories according to predominate cells B-lymphoblastic or T lymphoblastic leukemia ***(1)***.

This group of neoplasms shares morphological and immune-phenotypic levels, however, they are highly heterogeneous at the genetic level, which is thought to have an impact on the treatment and prognosis of the disease. Increasing scientific efforts are paid to study this heterogeneity to provide insights to improve treatment outcomes by demonstrating more disease subsets with clinical significance, new markers for risk stratification and minimal residual disease, germline polymorphisms that help to personalize the treatment strategies, and find new therapeutic targets ***(2)***.

Over the last five decades, the management of acute lymphoblastic leukemia has been improved converting ALL from a nearly fatal disease to a disease with survival rates up to 90% ***(2)***.

Epidemiology

Acute lymphoblastic leukemia is the most prevalent malignancy in pediatrics worldwide, representing 28% of all childhood malignancies and 78% of leukemias. Except in some countries in the Equator region in Africa where Lymphoma is more common than leukemia. The overall incidence is 42 cases per million children, this incidence has increased over the past several decades, though recently it has shown some stability ***(3)***.

The disease is slightly more common in boys, and shows a peak of incidence between 1-4 years, with rates in this age group near 100 cases per million, some epidemiological studies show that the risk of disease increase by 2-4 times in children with affected siblings. There is some difference between ethnic groups and geographical areas in ALL incidence, which is higher in North Americans and Europeans, while Asians, Africans, and South Americans show a lower rate, Hispanics are showing the highest rates of ALL ***(3)***.

Some studies refer to this difference in socioeconomic position, showing a correlation between the risk of developing ALL and socioeconomic level (the peaks of social development and ALL go together). There is another possible hypothesis that ALL incidence is linked to increasing industrialization, which leads to increased exposure to chemical pollution inducing more somatic mutations. The 5-year overall survival rate (OS) is about 89% in children and decreases with age to reach 61% in young adults, an exception to this rule is infants less than one year old, for whom 6-year OS is 58.2% and there is no improvement in this age survival until now. However, overall survival and mortality rates do not accurately describe the disease burden on public health, as there is an increase in the risk of cognitive deficit, congestive heart failure, debilitating chronic conditions, obesity, second neoplasms, and early mortality among ALL survivors ***(4)***.

In Egypt, there are 4 new cases per 100.000 children per year diagnosed with ALL in the National Cancer Institute (NCI), Cairo University. While the incidence of leukemia at Children's Cancer Hospital Egypt (CCHE) was 27.6%, The 5-year overall survival of children with ALL aged 0-18 years at CCHE during the diagnosis period 2007-2017 was 79.1% ***(5)***.

Causes and Risk Factors

Even with the exceptional advances in molecular studies and the increased understanding of many cancers' genomic landscape, the definitive cause of ALL and its pathogenesis are still unclear. It is thought that ALL is multifactorial, in which there is an interplay between multiple environmental and genetic factors ***(6)***.

Genetic predisposition

Genetic predisposition to ALL has been proved by many lines of evidence, including the presence of constitutional syndromes and familial cancer syndromes associated with increased incidence of ALL, also some germline mutations and silent polymorphisms have been found to have an impact on ALL susceptibility ***(7)***.

Genetic syndromes

There are many genetic syndromes associated with an increase in risk of developing ALL, some studies were done in the UK for example showing that 2.6% of children with leukemia were known to have a genetic condition, the most common one is Down syndrome (DS), DS children have 33-fold increase in incidence of ALL specially B-ALL. Other genetic syndromes that may associate higher ALL susceptibility are DNA damage repair defects: e.g. Fanconi anemia, ataxia telangiectasia, Nijmegen breakage syndrome, and Bloom syndrome and cell cycle and differentiation defects: including neurofibromatosis type 1, also Familial cancer syndromes as Li-Fraumeni syndrome which associated with an increase in the risk of certain types of cancer including ALL ***(8)***.

Genetic mutations

Studies showed that many somatic or germline genetic mutations associated with ALL occur in genes coding for some antiproliferative factors and other key pathways in lymphoid development, leading to disrupted cell cycle regulation and apoptosis. Examples of germline mutations associated with ALL are PAX5, and TP53 germline mutations, which are considered examples of familial ALL. Translocations are also common, resulting in chimeric fusion genes between proto-oncogene and some transcription factors e.g. immunoglobulin gene enhancers on chromosome 14, these chimeric genes have an oncogenic effect by disruption of cellular pathways of differentiation and proliferation ***(9)***.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) in coding or non-coding DNA were found that they affect personal susceptibility to ALL and have prognostic and therapeutic implications. Genome-wide association Studies (GWAS) have identified some SNPs associated with an increase in the risk of ALL, for example, ARID5B, IKZF1, CEBPE, ETV6, CDKN2A, PIP4K2A-BMI1, and GATA3, the latter is associated with an increase incidence of BCR-ABL1 subtype of ALL in particular, giving an example on SNP can be associated with a molecular subtype of ALL ***(10)***.

Epigenetics in ALL

Epigenetic regulations control chromatin structure and normal functions in all body including hematopoietic cells, so disruption of one of these mechanisms may occur during leukemogenesis. The most important epigenetic mechanisms are DNA methylation, Histone modification, and non-coding RNAs ***(11)***.

DNA methylation

It is the most obvious epigenetic regulation mechanism, it controls cellular identity and differentiation by methylation of cytosine at the CpG island **,** some studies show a significant difference in methylation patterns in ALL patients in comparison with healthy controls, also methylation signature differs in between different ALL subtypes, which corresponds to different gene expression profiles ***(12)***.

MicroRNAs

They are small noncoding RNA molecules, responsible for posttranscriptional regulation of gene expression, they are key regulators in hematopoiesis and highly involved in leukemia pathways, there are at least 32 miRNAs known to have a role in ALL pathology and prognosis. There is reciprocal regulation between both miRNAs and DNA methylation, miRNAs control methylation through DNA methyltransferase or methylation-targeted proteins, while defects in methylation of miRNAs can occur frequently in human cancers, including ALL ***(13)***.

Histone modification

Modification of histones is done by either methylation, acetylation, or phosphorylation. Mutations associated with modification in histones are frequent with T-ALL rather than any pediatric malignancy, although some recent studies provide strong evidence of their role in the pathogenesis and prognosis of B-cell leukemias especially MLL rearranged leukemia ***(14)***.

Heritability and prenatal origin

Genetic susceptibility is an important factor in the development of ALL, not only individual's genetics, but also maternal ones may have a role, some researchers suggest that the mother's genotype can affect the baby's health during pregnancy by controlling how carcinogens or DNA-damaging substances are metabolized in her body, which in turn affect the degree of fetal exposure to carcinogens in the womb, but still no evidence on clear causative agents ***(9)***.The in-utero initiation of many subtypes of ALL has been well-established through twin studies and retrospective analysis of neonatal blood ***(15)***.

Molecular studies of identical twins concordant for ALL provide strong evidence that ALL starts in utero in one fetus and then is shared with the other one through the placental blood ***(7)***., for example, a study identified ETV6/RUNX1 translocation in twins who developed ALL almost at the same time and have nearly similar breakpoints in the gene ***(16)***.

Also, retrospective backtracking studies of dried blood spots on Guthrie's cards of children with ALL could identify cells harboring TEL-AML and hyperdiploidy genetic abnormalities. However, population studies showed that only a small minority of newborns harboring these defects develop ALL, which means that these genetic defects develop only preleukemic cells**,** not leukemic ones, and there are further infrequent events needed to produce ALL, this is known as multistep model of leukemogenesis which suggests that the development of ALL requires at least two different genetic hits the first of them occur prenatal. T-ALL is **an** exception, as most of the known genetic abnormalities associated with T-ALL are not seen sufficiently in neonatal blood spots ***(17)***.

Environmental factors

It is well-established that exposure to ionizing radiation even before birth is directly linked to ALL, however, studying the effect of other environmental factors for example viruses, chemical mutagens, and

chemical contamination of groundwater didn't show definitive causation. However, recent studies show that maternal exposure to pesticides may be associated with an increase in ALL risk. Also, there is recent evidence that exposure to common infections at an early age (by using Day-care attendance as a strong surrogate measure) may be a protective factor from childhood ALL, this is explained as delayed exposure leading to dysregulated immune response which may act as the second hit of leukemogenesis**.** Therefore, Greaves 'delayed infection' hypothesis assumed that common infections could have antagonistic (early exposure) or promoting (late exposure) effects on the risk of ALL ***(18)***.

Classifications of ALL

Acute lymphoblastic leukemias were classified at first according to morphological features, using the French-American-British (FAB) classification into 3 major subtypes (L1, L2, L3), these morphological features include cell size, cytoplasm, nucleoli, vacuolation and basophilia. While the more recent classifications classify according to a combination of morphological and cytogenetic profiles of leukemia blasts, aiming to better risk stratification and introduce new targeted therapies to improve prognosis. Currently, there are two parallel systems of classification International Consensus Classification (ICC) and WHO classification, the last update in WHO classification was in 2022: Hematolymphoid Tumors 5th edition. The main outline of these classifications is classifying ALL into B-ALL and T-ALL, B-ALL is further sub-classified according to WHO into B-ALL not otherwise specified and B-ALL with recurrent genetic abnormalities, the latter includes many subtypes ***(19)***.

Table (1): WHO Classification of Hematolymphoid Tumors, 5th edition: B-cell lymphoblastic leukemias/lymphomas**.**

B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with high hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with iAMP21
B-lymphoblastic leukemia/lymphoma with BCR::ABL1 fusion
B-lymphoblastic leukemia/lymphoma with BCR::ABL1-like features
B-lymphoblastic leukemia/lymphoma with KMT2A rearrangement
B-lymphoblastic leukemia/lymphoma with ETV6::RUNX1 fusion
B-lymphoblastic leukemia/lymphoma with ETV6::RUNX1-like features
B-lymphoblastic leukemia/lymphoma with TCF3::PBX1 fusion
B-lymphoblastic leukemia/lymphoma with IGH::IL3 fusion
B-lymphoblastic leukemia/lymphoma with TCF3::HIF fusion
B-lymphoblastic leukemia/lymphoma with other defined genetic abnormalities*

* Other defined genetic abnormalities: include B-ALL with DUX4, MEF2D, ZNF384, NUTM1 rearrangements, with IG::MYC fusion, and with PAX5alt or PAX5 abnormalities.

About 75% of childhood ALL patients have recurring chromosomal alterations either aneuploidy or translocations, which can be detected by methods varying from karyotyping which identifies large alterations to whole genome sequencing which detect cryptic changes in the entire genome. Identification of these abnormalities is crucial for optimum disease evaluation, risk stratification, and treatment planning. Up to 10% of childhood ALL is T-ALL however, B-ALL represents 90%, including 31% with B-ALL with high hyperdiploidy, 21% B-ALL with ETV6::RUNX1, 7% other cytogenetic abnormalities (e.g.: B-ALL with BCR::ABL1, ALL with KMT2A, nearhaploidy/low haploidy, B-ALL with TCF3::PBX1, B-ALL with TCF3::HLF and B-ALL with IGH::IL3), and 30% of B-ALL with not identified specific genetic abnormalities, termed "B-other" ***(19)***.

B-Lymphoblastic leukemia subtypes

ALL with high hyperdiploidy (51–67 chromosomes), which is the most common cytogenetic subtype in pediatric ALL, which is associated with favorable outcomes in children, especially in the presence of extra copies of chromosomes 4, 10, or 17 ***(20)***

Additional genetic abnormalities are common with hyperdiploidy supporting the multistep theory ***(20)***, for example, mutations in the RTK-RAS pathway and mutation in histone modifiers e.g. CREBBP**. ALL with hypodiploidy** in which chromosome number is less than 44 chromosomes. The 5th edition of WHO classification has approved further sub-classification of hypodiploid ALL into 3 subtypes: near haploid 24-31 chromosomes, low-hypodiploid 32-39 chromosomes, and high-hypodiploid 40-43 chromosomes. Near-haploid and low-hypodiploid are unfavorable in prognosis and show specific mutations, in near-haploidy: Ras mutations and deletions of IKZF3, while in low hypodiploidy associated with deletion of IKZF2 and near-universal TP53 alteration 50% of TP53 mutations associated with hypodiploid-ALL are germline in nature, suggest that this subtype is linked to Li-Fraumeni syndrome ***(21)***.

B-ALL with intrachromosomal amplification of chromosome 21 (iAMP21), and it is associated with poor prognosis of standard therapy. However, some studies show that intensification of therapy improves the diagnosis. This abnormality *is* defined by the presence of a total of five or more copies of the RUNX1 gene at least three of them on the same chromosome, to exclude hyperdiploidy. Notably, both copies of chromosome 21 are never lost in hypodiploid ALL, together with the high incidence of ALL in trisomy 21 (DS) and ALL with iAMP21 suggest that chromosome 21 has an important role in leukemogenesis however, the key driver gene is still unknown ***(22)***.

B-ALL with ETV6::RUNX1 rearrangement, also called TEL-AML1 rearrangement, was first described 20 years ago and considered one of the most common subtypes of ALL.This rearrangement occurs by t(12;21) translocation, leading to the fusion of the TEL gene (ETV6) at 12q13, which functions as a transcription regulator, with AML1 (RUNX1) at 21q22, which is thought to have an essential role in the regulation of many genes involved in hematopoiesis. The fusion creates a chimeric oncoprotein TEL/AML1 which leads to the activation of kinase or alteration of transcriptional regulations. This subtype has an excellent prognosis, it is usually associated with other genetic alterations which may act as cooperative mutations to produce ALL, eg deletion of the normal *ETV6* allele and focal deletion of PAX5 ***(23)***.

B-ALL with BCR::ABL1 fusion, which results in Philadelphia chromosome formation, although the Philadelphia chromosome is tightly linked to chronic myeloid leukemia (CML), it is found also in ALL, with incidence increasing by the increase in age, until reaching the peak of age 35-50 years. It results from a reciprocal translocation between proto-oncogene ABL on chromosome 9 and the BCR gene on chromosome 22, leading to activation of the ABL tyrosine kinase in hybrid Bcr/Abl oncoproteins, which is considered essential for the development of leukemia by altering signaling pathways regulates cell survival and proliferation and self-renewal of stem cells. Generally, the presence of BCR-ABL predicts poor outcomes, however, the prognosis had been significantly improved by the addition of tyrosine kinase inhibitors (TKAs) ***(23)***.It is commonly associated with IKZF1 haploinsufficiency, which is found deleted in over 80% of BCR-ABL1 ALL patients, in many studies, IKZF1 mutations are associated with poorer prognosis and higher risk of relapse ***(23)***.

B-ALL with BCR::ABL1-like, or Ph-like, which is a subtype of ALL shows a gene expression profile similar to BCR::ABL1 ALL with an absence of Philadelphia chromosome translocation ***(24)***.

B-ALL with TCF3::PBX1 rearrangement, which is a chromosomal translocation between the transcription factor 3 gene (TCF3) at chromosome 19 and PBX1 gene at chromosome 1 ***(24)***.

The prognosis of this subtype used to be poor, however, the survival rates have been improved recently with the intensification of therapy ***(24)***.

B-ALL with TCF3::HLF fusion, Both the TCF3 at chromosome 19 and HLF genes at chromosome 17 encode transcription factors. This fusion is a rare translocation t(17;19), however, all the reported cases of TCF3-HLF chimera were in B-ALL patients, associated with dismal prognosis ***(22)***.

B-ALL with KMT2A rearrangement, KMT2A gene also called mixed linage leukemia (MLL) gene is located at chromosome 11, it is most common in infants less than 1 year old representing 80%, this

rearrangement also occurs in AML, so is called mixed linage leukemia gene. This subtype shows sensitivity to nucleotide analogs e.g., cytosine arabinoside (araC) ***(24)***.

B-ALL with IGH::IL3 rearrangement, ALL with translocation t(5;14), considered an exceptional in association with marked eosinophilia, this translocation juxtaposes the IGH enhancer located on 14q32 to the IL3 gene on 5q31, lead to formation of fusion protein stimulate production of interleukin-3 (IL3) which in turn induces the maturation and release of eosinophils in the bloodstream ***(25)*.**

T-Lymphoblastic leukemia

Classification of T-ALL is complex and the definition of T-ALL subtypes shows high variation in recent literature, WHO 5th edition continues in classify T-ALL according to immunophenotype into early T-precursor lymphoblastic leukemia/lymphoma (ETP-ALL) and T-ALL NOS. ETP-ALL shows immunologic markers and gene expression profiles similar to double negative 1 thymocytes, which can differentiate into T-linage or myeloid linage but not B-cell linage. The prognosis of T-ALL is generally poor, although it shows some recent improvement with the intensification of therapy, it is still lower than the prognosis of B-ALL, reflecting the need for more molecular studies and novel therapies for the high-risk T-ALL ***(26)***.

As for genetic lesions in T-ALL, chromosomal translocations and dysregulated oncogenic pathways are found in about 50% of cases but unlike B-ALL the prognostic significance of any of them is still unclear yet they are not used in risk stratification yet. One of the most important oncogenes in T-ALL is NOTCH1, as the NOTCH1 signaling pathway is abnormally activated in more than 60% of T-ALL cases. NOTCH1 is a member of a family of heterodimeric transmembrane proteins, which is required in multiple stages of normal T lymphocyte development. Leukemic transformation can result from NOTCH1 activation in the presence of other cooperating mutations such as deletion of tumor suppressor gene CDKN2A (which is found in 70% of patients with T–ALL), so NOTCH1 inhibition is presented as a potential target in T-ALL therapy ***(27)***.

Clinical picture

Clinical presentations of ALL are usually nonspecific, representing a challenge for early diagnosis of the disease, the onset of the disease is usually sudden; however, the symptoms might take anywhere from a few days to several months. Pathophysiology of the disease can be explained by being a lymphoproliferative disease, in which immature blasts replace normal hematopoietic cells in the bone marrow and infiltrate extramedullary organs ***(28)***.

Medullary involvement can cause the most prevalent clinical signs of ALL, leading to bone marrow failure syndrome, As a result, the patients may develop recurrent infections, pallor, bleeding, petechia, purpura, fatigue, muscle pain, anorexia, and pallor. ALL patients are considered immune compromised regardless of their white blood cell count, as neutropenia which is defined as a decrease in the absolute count of neutrophils below 500 and is common even with the presence of high WBCs, leads to infection and febrile illness. The earliest presenting symptoms in more than 38% of children with ALL are those of the musculoskeletal system, including limping, pain in the bones or joints, and bone fracture, due to leukemic infiltration in the periosteum ***(29)***.

Infiltration of extramedullary organs is common; for example, hepatosplenomegaly, which occurs in 68% of patients and is often asymptomatic, and lymphadenopathy, which is typically painless. Involvement of the skin, kidneys, eyes, liver, and CNS can also occur. Increase intracranial tension occurs secondary to CNS infiltration, leading to headache, confusion, projectile vomiting, and neck stiffness. Painless unilateral testicular mass is seen in ~2% of boys at diagnosis , both Testes and CNS are considered sanctuary sites for leukemia cells from which Extramedullary relapse originates. An anterior mediastinal mass may present in 10% of patients with T-ALL due to infiltration of the thymus gland, which may lead to superior vena cava syndrome; including pleural effusions and respiratory distress ***(30)***.

None of these symptoms are pathognomonic for leukemia, however, an underlying cancer condition should be taken into consideration if any of these common symptoms are prolonged than usual ***(30)***.

Diagnosis of ALL

Initial Laboratory Evaluation

When ALL is first suspected, the initial investigations carried out are complete blood count (CBC) and blood film. These tests are important in being rapid, and affordable with a high degree of sensitivity. CBC shows pancytopenia due to bone marrow infiltration. Although most patients tend to be neutropenic, the total white blood cell count is elevated due to the presence of millions of circulating blasts, these blasts are evident on the blood film. The suspicious clinical context together with any abnormality of the complete blood count and/or film microscopy should be followed by bone marrow aspirate. In ALL bone marrow shows hypercellularity, with high leukemic blasts of more than 20%. The blasts are further described by morphology, immunophenotyping, and cytogenetic analysis for further characterization of ALL subtypes and guiding treatment strategy. Cerebrospinal fluid (CSF) evaluation should be performed at the time of diagnosis, as CNS involvement may occur even with the absence of neurological symptoms. Evaluation of blood chemistries is important to exclude tumor lysis syndrome, which is evident with hypocalcemia, hyperkalemia, hyperphosphatemia, elevated lactate dehydrogenase (LDH), hyperuricemia, and elevated creatinine ***(31)***.

Morphology

In ALL, lymphoblasts may have a wide variety of sizes and shapes. Contrary to the WHO classification, which considers cytogenetic and immunologic characteristics, the FAB classification emphasizes the presence of subgroups of precursor lymphoblasts: L1, which is more prevalent in children, L2, which are more prevalent in adults, and L3, which represent the more developed subtypes of B-cells. Immunophenotyping is usually required for confirmation since B-cell precursors and T-cell precursors cannot be consistently distinguished from each other based just on morphology ***(32)***.

Immunophenotyping

Immunophenotyping identifies and quantifies cluster differentiation (CD) antigens through fluorochrome-labeled monoclonal antibodies. CD antigens are a group of certain cytoplasmic and surface proteins expressed by blood cells, every cell lineage at a specific level of differentiation has a unique set of expression of the CD antigens. Using immunophenotyping ALL can be classified into clinically significant subgroups, which are not only used in as a diagnostic tool but also provides prognostic insights and helps in offering the most specific treatment to the patients. Based on the blasts reactivity pattern with a panel of lineage-associated antibodies, immunophenotype classifies ALL into B-lineage phenotype, which is positive for B cell markers: CD19, CD20, CD22, TdT, cytoplasmic CD79a, CD34 and CD10, the pattern of expression of these antigens reflects the degree of maturation of B cell, leasing to further subclassification into early pre B (pro-B), pre-B, transitional (or late) pre-B and (mature) B-ALL (***33)***.

While T-lineage ALL express T cell markers which are cytoplasmic CD3and CD7 plus CD2 or CD5, this lineage is further subdivided into early, mild, or late thymocyte differentiation (***33)***.

The immunophenotype of leukemic cells is often correlated with biological features used in subclassifications, including specific genetic abnormalities, for example, The presence of *ETV6- RUNX1* is reported to be correlated with a CD27-positive/CD44-negative immunophenotype, while *TCF3-PBX1*-positive BCP-ALL patients tend to express the cytoplasmic μ chain-positive pre-B ALL immunophenotype with complete absence of CD34 (***33)***.

Cytogenetic studies

Identifies specific genetic abnormalities which play a critical role in leukemogenesis and have important prognostic and therapeutic implications, by using RT-PCR, FISH/multiplex ligation-dependent probe amplification, and flow cytometry. These abnormalities include leukemia-specific translocations, submicroscopic chromosomal abnormalities, and cellular DNA content. The current diagnostic techniques may be replaced with genome-wide analysis after the increase in time and cost-effectiveness ***(7)***.

Management of ALL

Treatment of childhood ALL has achieved great success over the last few decades through the use of risk-directed therapy, which lead to an improvement in survival rates to exceed 90% in developed countries, however, low/middle-income countries still achieve less results. This success is largely due to the discovery

of the effective dosages and regimens of chemotherapeutic medicines that have been accessible for decades, rather than the development of new treatments ***(34)***.

Risk Stratification

Risk assessment is important to ensure that the very intensive treatment is given only to patients who need it being high-risk cases and avoid unnecessary complications of intensive chemotherapy for the low-risk group. The current clinical protocols use a combination of clinical and biological factors and the response to early treatment ***(35)***

Treatment

Currently, treatment of ALL depends on chemotherapy, central nervous system (CNS) prophylaxis, and Hematopoietic stem cell transplantation (HSCT) ***(36)***.

Chemotherapy

Treatment of ALL involves three steps of multidrug chemotherapy: induction of remission, consolidation (intensification), and maintenance therapy to eliminate any residual leukemia ***(35)***, treatment usually takes 2-2.5 years ***(7)***.

The goal of induction of remission is to eliminate more than 99% of the leukemic load and to return hemopoiesis to normal within about 4 to 6 weeks **.**For most study groups, this goal is achieved with the use of a three-drug approach: vincristine, a glucocorticoid, and anthracycline to which asparaginase may be added as a fourth drug. After induction, consolidation is used to eliminate any submicroscopic illness that remains after complete remission has been achieved. It varies in length and severity according to the used protocol and the level of risk of patients and usually lasts between 6 to 9 months. This phase involves a combination of different agents to increase synergism and decrease the incidence of resistance, for example, mercaptopurine, thioguanine, methotrexate, cyclophosphamide, etoposide, and cytarabine. Following consolidation therapy, patients continue on maintenance therapy, which usually lasts 1-2 years, and usually includes a combination of daily administration daily 6-mercaptopurine, and weekly methotrexate ***(37)***.

CNS directed therapy

CNS therapy is used to prevent CNS relapse via the elimination of leukemic cells from CNS as it cannot be easily accessed with systemic chemotherapy due to the blood-brain barrier, which is generally given to all patients during ALL therapy. CNS-directed therapy may include intrathecal therapy (i.e. intrathecal methotrexate), cranial irradiation, and systemic chemotherapy may be added. Due to the significant risk of late neurocognitive sequelae, endocrinopathy, and secondary malignancies, cranial irradiation has recently been largely replaced by other therapy methods, and currently is only kept to those with high-risk disease or CSF-positive for leukemia at the time of diagnosis ***(38)***.

Hematopoietic stem cell transplantation (HSCT)

HSCT from peripheral blood or bone marrow is an important post-remission strategy, but the curative potential of HSCT must be balanced against the disadvantages. Patients with poor response to chemotherapy, induction failure, early bone marrow relapse (less than 18 months from diagnosis) or recurrent bone marrow or CNS relapses, and unfavorable cytogenetics including Philadelphia chromosome are thought to benefit from HSCT. Better results can be obtained from patients who receive transplants after being negative for MRD. Traditionally, a matched sibling has been the best donor, however, recent promising studies show success with alternative donor sources ***(40)***.

Novel therapies

Providing individualized treatment regimens by including precisely tailored therapies to subcategories of disease often defined by patient genomics is one of the current areas of scientific research; this concept is known as precision medicine. The use of precision medicine in cancer therapy has been greatly enhanced by the identification of numerous molecular abnormalities that are connected to cancers. These findings lead to the demonstration that each cancer has a unique genomic signature, and features unique to each tumor, with some additional characteristics that are shared by various cancers, as well as identification of pathways altered in some tumors. Along with novel immunotherapies, several targeted treatments have been developed and several of them have demonstrated excellent results. An important example is the use

of tyrosine kinase inhibitors (TKI) like imatinib in patients with BCR-ABL1 fusion oncoprotein with is found in chronic myeloid leukemia (CML) and Philadelphia-positive ALL ***(40)***.

Ph+ ALL has previously been linked to unfavorable prognosis, with a five-year survival rate of fewer than 20%. The use of TKIs with cytotoxic chemotherapy has improved response rates in both pediatric and adult populations, which may lead to minimizing the need to HSCT in those patients soon, especially in children ***(40)***.

Immunotherapy is another innovative treatment option by using antibody-based therapy, for example, blinatumomab which is a bispecific T-cell engager, which utilizes the patient's own cytotoxic T cells to attack and induce lysis of CD19-expressing leukemic cells, many studies have proved its effectiveness in inducing complete remission and reduction of MRD in both adult and pediatric ALL, with better-tolerated side effects than conventional chemotherapy ***(41)***.

Minimal residual disease

A patient's total number of leukemia cells at the time of diagnosis is around 10^{12}-10^{13}. After 4 weeks of chemotherapy, most patients attain complete remission (CR), which is determined by a blast cell count of less than 5% in the bone marrow, however, this only indicates that the number of leukemia cells is below the sensitivity level of the traditional morphological examination methods, not that they have completely disappeared from the body. Many cancerous cells may still be present in the patient at this time, they represent the minimal residual disease (MRD), which needs to be detected by using more sensitive methods ***(42)***.

The idea of MRD detection techniques is the isolation and quantification of a leukemia cell-specific marker. The currently used markers are either immunophenotypic markers isolated by flow cytometry or the more sensitive PCR assay which depends on genetic markers ***(7)***.

Relapsed ALL

Despite intensive treatment, the incidence of relapse is still high, with about 15-20% of ALL children. Relapse represents the emergence of a clonal cell population that was not entirely eradicated by. Bone marrow is the most common site, other sites include CNS and the testicles, the majority of ALL relapses occur during therapy or the first 2 years following treatment completion, however, some relapses have been known to happen even 10 years after diagnosis. The prognosis of these individuals is mostly influenced by the site and the interval between diagnosis and relapse, also certain genetic subtypes may have an effect. In clinical practice, individuals can be divided into two categories based on these factors: standard or high-risk start relapsed ALL. relapsed patients have a high risk of subsequent treatment failure, however, the best possible curative treatment is HSCT following a second complete remission ***(43)***.

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