INTRODUCTION

Childhood acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is a malignant clonal disorder that originates in a single B- or T-lymphocyte progenitor cell. It results in proliferation and accumulation of blast cells in the bone marrow (BM), which in turn leads to suppression of hematopoiesis and disruption of the normal BM cell distribution and finally appears in the peripheral blood (PB). This manifests as anemia, thrombocytopenia and neutropenia. Lymphoblasts can also accumulate in various extramedullary sites especially the liver, spleen, lymph nodes, meninges, gonads and thymus [(1)].

Epidemiology:

Worldwide, ALL is the most common cancer diagnosed in children and represents approximately 25% of cancer diagnoses among children younger than 15 years. ALL is the main subtype among childhood leukemias representing approximately 80%, with a sharp peak observed among children aged from 2 to 5 years [(2, 3)]. In adults, however ALL represents about 20 % of acute leukemias [(4)]. Up to 85% of childhood ALLs are of the precursor B-cell type and the remaining 15% are of the T-ALL type [(5, 6)].

In Egypt, according to the National Cancer Registry (NCR), leukemia is the leading cause of malignancy in children, constituting 35.6% of cases of childhood cancer diagnosed annually [(7)]. In 2001, according to International Classification of Childhood Cancer (ICCC), the incidence rate of childhood leukemias in Egypt in children less than 15 years is 31.9 per million populations [(8)], compared to 26 per million populations in the United States according to the National Cancer Institute's SEER program [(9)].

According to the statistics of the National Cancer Institute in Egypt from 2002 till 2010, pediatric leukemias represented 63.8% of malignancies of lymphohemopoietic system. In both Egypt and western countries the comparative relative frequency of ALL incidence among children (<15 years) was found to be higher (44.8%) than in adolescents (15 to <20 years) (18.8%). Moreover, a comparison between Egypt and western countries as regard disease relative frequency among both previous age groups has revealed that among children (<15 years); the frequency in Egypt was (20.3%) when compared to western countries which was (24.5%), however in adolescents (15 to <20 years); the frequency in Egypt was found to be (12.4%) when compared to western countries which was (6.4%) [(2)].

ALL occurs more frequently in males than in females and three times more frequent in whites than in blacks [(10, 11)]. Children with certain genetic syndromes (e.g. Down syndrome, Neurofibromatosis type 1, Bloom syndrome and ataxia telangiectasia) are at higher risk of developing ALL [(12,13)], especially those having down syndrome show 10 to 30 folds increased risk of developing ALL [(14)].

Etiology and pathogenesis:

Initiation and progression of ALL are driven by successive mutations that alter the cellular functions including; an enhanced ability of self-renewal, loss of control of normal proliferation, a block in differentiation and an increased resistance to death signals (apoptosis) [15,16]. Epidemiological and case control studies have found more than twenty candidate exposures that contribute to childhood ALL [5]. Some environmental agents have been implicated in the induction of ALL in some patients and are of public concern, especially ionising, non-ionising (e.g electromagnetic field) radiations and chemical mutagens. Ionising radiation is an established causal exposure for childhood ALL, as evidenced by the impact of the 1945 atomic bombs in Japan. Infection was also from the first suggested causal exposures for childhood ALL [17]. However, in most cases no etiologic factors were identified. In the favored theory, leukemogenesis reflects the interaction between host pharmacogenetics (susceptibility) and environmental factors, a model that requires confirmation in well-designed population and molecular epidemiologic studies [1].

Clinical presentation:

The clinical presentation of ALL is variable. Very rarely ALL produces no signs or symptoms and is detected during routine examination [18]. The presenting features generally reflect the degree of marrow failure and the extent of extramedullary spread [1].

Symptoms may appear insidiously or acutely. Fever is a common complaint found in about half of the patients, which is often induced by pyrogenic cytokines released from leukemic cells. In these patients, fever resolves within 72 hours after the start of anti-leukemic therapy. Fatigue and lethargy are also common complaints due to anemia. More than 25 percent of patients, especially young children may have bone pains, arthralgia or an unwillingness to walk because of leukemic infiltration of the periosteum, bone or joint or because of expansion of the marrow cavity by leukemic cells. Headache, vomiting, altered mental function, oliguria and anuria are less common presenting features. Occasionally, patients may present with a life-threatening infection or bleeding (e.g. intracranial hematoma). Intracranial hemorrhage occurs mainly in patients with an initial leukocyte count greater than 400×10^9/L.

Among the frequently evident findings are pallor, petechiae and ecchymosis in the skin and mucous membranes due to thrombocytopenia, in addition to bone tenderness which may result from leukemic infiltration. Liver, spleen, and lymph nodes are the most common sites of extramedullary involvement and the degree of organomegaly is more pronounced in children than in adults. A bulky anterior mediastinal mass can compress the great vessels and trachea and possibly lead to the superior vena cava syndrome, this is more common among T-ALL patients. Patients with this syndrome present with cough, dyspnea, orthopnea, dysphagia, stridor, cyanosis, facial edema, increased intracranial pressure and sometimes syncope [1, 19].

Classification:

French-American-British (FAB) Co-operative Group classification:

In the past ALL lymphoblasts were classified using the FAB criteria, which was based primarily on the microscopic appearance of the leukemic cells as seen on Wright-Giemsa-stained smears and cytochemistry into; L1, L2 and L3 subtypes[20,21]:

L1 subtype: It is the most common subtype. Leukemic blast cells are small with homogeneous chromatin, regular nuclear shape, small or absent nucleolus and scanty cytoplasm.

L2 subtype: It is less common than L1 subtype. Leukemic blast cells are large and heterogeneous with heterogeneous chromatin, irregular nuclear shape and the nucleolus is often large and with more abundant cytoplasm.

L3 subtype: It is the least common subtype. Leukemic blast cells are large and homogeneous with multiple nucleoli, moderate deep blue cytoplasm and cytoplasmic vacuolations that often overlie the nucleus (most prominent feature).

FAB classification resulted in great improvement in separation between ALL, AML and MDS. However, it is no longer used because its inability to differentiate B-ALL from T-ALL and its inability to determine the stage of blast maturation. Also, the subjective nature of this classification remains an issue as it depends solely on morphological criteria to classify acute leukemia[22].

WHO classification:

Before 2008, World Health Organization (WHO) classified B-lymphoblastic leukemia as precursor B-lymphoblastic leukemia and this terminology is still frequently used in the literature of childhood ALL to distinguish it from mature B-ALL; now termed Burkitt leukemia, which requires different treatment than has been given for precursor B-ALL. However, WHO 2008 (Table 1) classified the L1 and L2 subtypes of ALL as Precursor Lymphoid Neoplasms including; (a) B-lymphoblastic leukemia/ lymphoma not otherwise specified, (b) B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities and (c) T-lymphoblastic leukemia/ lymphoma. The L3 subtype of ALL was included in the group of mature B-cell neoplasms as the subtype Burkitt lymphoma/leukemia. The characteristic features of each are as follows [21, 23]:

B-Lymphoblastic leukemia/lymphoma, not otherwise specified:

It comprises 75% of cases in children less than 6 years of age. BM is involved in almost all cases while extramedullary involvement is frequent; CNS, lymph nodes, spleen, liver and testes are commonly involved. Total leucocytic count (TLC) may be normal, increased or decreased. Typically blasts are small with scant cytoplasm, indistinct nucleoli and condensed chromatin. Less commonly larger blasts with light blue to blue gray occasionally vacuolated cytoplasm with round, irregular or convoluted nuclei and dispersed chromatin and multiple nucleoli are also seen. Cytochemistry shows negativity for myeloperoxidase (MPO) and sudan black B (SBB) stains, while periodic acid schiff

(PAS) may be positive. Immunophenotyping shows CD19, cyt CD79a, cyt CD22, CD10, CD22, CD24 and terminal deoxy-nucleotide transferase (TdT) positivity.

B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities:

B-lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2) BCR-ABL 1: This subtype is more common in adults. Approximately 25% of adult ALL and 2-4% of childhood ALL belong to this subtype. It carries the worst prognosis.

B-lymphoblastic leukemia/lymphoma with t(v;11q23): Characterized by mixed-lineage-leukemia (MLL) gene translocation and it is usually CD19+ve, CD 10–ve and CD24 –ve.

B-lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) TEL-AML 1: It is common in children, comprises 25% of cases of B-ALL and is rare in adults. It is CD19+ve, CD 10+ve, most often CD 34+ve and carries favorable prognosis. Cure occurs in more than 90% of children.

B-lymphoblastic leukemia/lymphoma with hyperdiploidy: Karyotype often shows > 50 chromosomes, typically without translocations or structural alterations. This subtype is common in children, comprises 25% of cases of B-ALL. It is CD19+ve and CD 10+ve. Trisomies carry the best prognosis.

B-lymphoblastic leukemia/lymphoma with hypodiploidy: Usually <46 chromosomes, accounts for 5% of B-ALL cases. Both children and adults are equally affected. It is CD 19+ve and CD10+ve.

B-lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) IL3-IGH: It is a rare subtype, accounts for 1% of B-ALL cases. Both children and adults are affected. This subtype may have increased circulating reactive eosinophils and blasts are usually sparse .

B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3) E2A-PBX1(TCF3-PBX1): It is relatively common in children, accounting for 6% of cases of B-ALL. It is CD 10+ve and CD19+ve. This subtype carries poor prognosis.

T-lymphoblastic leukemia/lymphoma:

T-lymphoblastic leukemia/lymphoma (T-LBL) comprises 15% of childhood and 25% of adult ALL and comprises 85-90% of all lymphoblastic lymphomas. It is more frequent in adolescent males and BM is involved in almost all cases. Extramedullary sites include thymus, lymph nodes, skin, tonsils, liver, spleen, CNS and testes. It commonly presents with high TLC and large mediastinal mass. BM hematopoiesis may be relatively spared. Morphologically blasts may be indistinguishable from B-ALL blasts. Blasts are heterogeneous, of medium size and with high nucleocytoplasmic ratio, but considerable range of small blasts with very condensed nuclear chromatin and inconspicuous nucleoli may also be seen. In majority of cases large blasts with fine chromatin, prominent nucleoli and round to irregular convoluted nuclei are seen. The cytochemistry may show focal or polar acid phosphatase (ACP) positivity. Abnormal Karyotype is seen in 50-70% cases. Patients with this subtype are at more risk of induction failure, early relapse and isolated CNS relapse [(21)].

Table (1): WHO 2008 classification of acute lymphoblastic leukemia (ALL) [23]

Precursor lymohoid neoplasms
B-cell lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;qll.2); *BCR-ABL1*
B-cell lymphoblastic leukemia/lymphoma with t(v;llq23); *MLL* rearranged
B-cell lymphoblastic leukemia/lymphoma with t(12;21)(pl3;q22); *TEL-AML1 (ETV6-RUNX1)*
B-cell lymphoblastic leukemia/lymphoma with hyperploidy
B-cell lymphoblastic leukemia/lymphoma with hypoploidy (hypodiploid ALL)
B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
B-cell lymphoblastic leukemia/lymphoma with t(l;19)(q23;pl3.3); *E2A-PBX1 (TCF3-PBX1)*
B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
T-cell lymphoblastic leukemia/lymphoma

Immunological classification:

Precursor B-ALL is defined immunologically by the expression of HLA-DR, TdT, CD19, CD79a, CD22 and CD20. Approximately 90% of precursor B-ALL cases express the CD10 (formerly known as common ALL antigen [CALLa]) [24]. (Table 2)

Table (2): Immunological classification of acute lymphoblastic leukemia according to the European Group for the Immunological Characterization of Leukemias (EGIL) proposal (Bene et al. 1995)

B-lineage ALL (CD19+ and/or CD79a+ and/or CD22+)	
Pro-B-ALL (B-I)	No expression of other differentiation B-cell antigens
Common B-ALL (B-II)	CD10+
Pre-B-ALL (B-III)	Cytoplasmic IgM+
Mature B-ALL (B-IV)	Cytoplasmic or surface kappa or lambda+
T-Lineage ALL (cytoplasmic/membrane CD3+)	
Pro-T-ALL (T-I)	CD7+
Pre-T-ALL (T-II)	CD2+ and/or CD5+
Cortical-T-ALL (T-III)	CD1a+
Mature T-ALL (T-IV)	Membrane CD3+, CD1a-

Precursor B-ALL is immunologically classified into three subtypes:

a. **Pro-B-ALL (also termed pre-pre-B-ALL), CD10 negative and no surface or cytoplasmic immunoglobulin (Ig):** found in approximately 9-11% of patients. It is the most common immunophenotype seen in infants and is often associated with t(4;11) [25].

b. **Common precursor B-ALL (CD10 positive and no surface or cytoplasmic Ig):** found in approximately three quarters of patients and has the best prognosis. These patients usually show favorable cytogenetics [26].

c. **Pre-B-ALL (presence of cytoplasmic Ig):** Leukemic cells contain cytoplasmic Ig (mainly IgM), which is absent in common ALL, otherwise it is identical to common ALL with respect to expression of all other cell markers. It represents 20-25% of childhood ALL. 25% of patients with pre-B-ALL have the t(1;19). Sometimes it is associated with t(9;22) [27,28].

Approximately 2% of children with ALL present with mature B-cell leukemia (surface Ig expression, generally with FAB L3 morphology and a translocation involving the C-MYC gene), also called Burkitt leukemia. CD10 may be present and in rare cases TdT is expressed. The treatment for mature B-ALL is based on therapy for non-Hodgkin lymphoma and is completely different from that for precursor B-ALL. Rare cases of mature B-cell leukemia that lack surface Ig but have L3 morphology with C-MYC gene translocations should also be treated as mature B-cell leukemia [29,30].

Cytogenetic and molecular abnormalities of precursor B-ALL:

Approximately 75% of childhood ALL cases harbor recurrent genetic abnormalities, including aneuploidy or structural chromosomal arrangements, which can be detected by conventional karyotyping and fluorescence in situ hybridization (FISH). The highest frequency translocations found in childhood B-ALL are: t(9;22) (q34;q11), t(12;21) (p13;q22), hyperdiploidy, and translocation t(4;11) (q21;q23) in infants. Other recurrent cytogenetic abnormalities include hypodiploidy and translocation t(1;19) (q23;p13) (Figure 1).

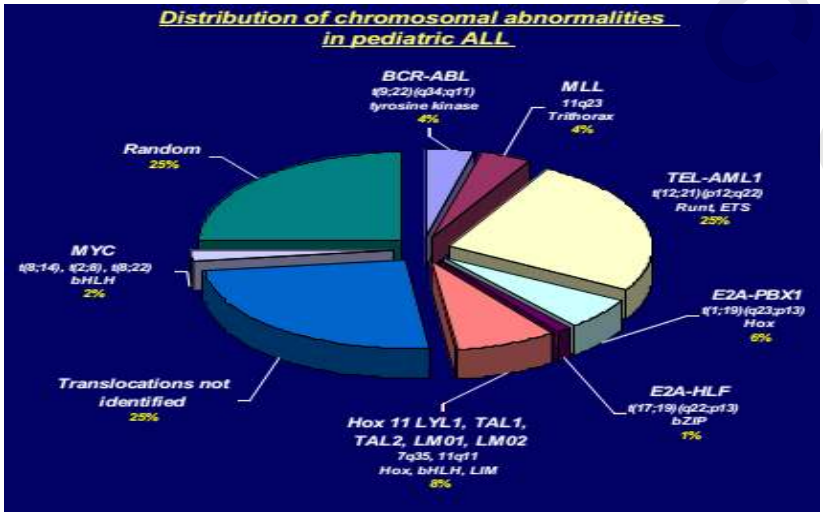


Figure (1): Distribution of chromosomal abnormalities in pediatric ALL. [32]

Aneuploidy:

Hyperdiploidy: (51–65 chromosomes) is one of the most common cytogenetic abnormalities. It is seen in 25-30% of childhood B-ALL, with the highest frequency from 1 to 4 years. High hyperdiploidy is characterized by a non random gain of chromosomes, including + X, +4, +6, +10, +14, +17, +18, and +21 .It indicates a good prognosis [33-35].

Hypodiploidy: is characterized by fewer than 46 chromosomes and is seen in 5-8% of total B-ALL cases. The majority of hypodiploid B-ALL contain 45 chromosomes. The remainder of hypodiploidy cases are much rarer and include; High-hypodiploid (40–44 chromosomes), Low hypodiploid (33–39 chromosomes) and near-haploid (24–29 chromosomes). In general, hypodiploidy with less than 40 chromosomes confers a poor prognosis [36].

Recurrent translocations:

t(12;21)(p13;q22) [ETV6-RUNX1(TEL-AML1)]: The most common chromosomal rearrangement in B-ALL is t(12;21)(p13;q22), encoding for ETV6-RUNX1 (TEL-AML1)[31]. It occurs in 25% of children with B-ALL and confers an excellent prognosis [37, 38].

t(1;19)(q23;p13) [TCF3-PBX1 (E2A-PBX1)]: The t(1;19)(q23;p13) rearrangement and its unbalanced variant der(19)t(1;19)(q23;p13) are commonly seen in B-ALL [31]. This translocation occurs in 6% of childhood B-ALL and is associated with poorer outcomes [36].

t(9;22)(q34;q11) [BCR-ABL1] (Philadelphia chromosome): The t(9;22)(q34;q11) or 'Philadelphia chromosome' (Ph + B-ALL), encodes for the fusion gene BCR-ABL1. This translocation is present in 3-5% of childhood B-ALL cases and is associated with; older age, higher leukocyte count and more frequent CNS involvement at the time of diagnosis. The translocation fuses the 5' sequence of the breakpoint cluster region (BCR) on chromosome 22 to the 3' sequence of the Abelson (ABL1) gene on chromosome 9. The resultant oncoprotein is a constitutively active non receptor tyrosine kinase, responsible for leukemogenesis. In childhood B-ALL fusion genes are created by breaks in the minor BCR (m-bcr) area which encodes for a 190 kDa fusion protein (p190) [23, 36].

MLL gene rearrangements: Mixed-lineage-leukemia (MLL) gene rearrangements at 11q23 are present in 80% of all infant B-ALL cases and 10% of childhood B-ALL [36].

The most common gene rearrangements include; t(4;11)(q21;q23) encoding MLL-AFF1(AF4), t(9;11)(p22;q23) encoding MLL-MLLT3(AF9), t(11;19)(q23;p13.3) encoding MLL-ENL and t(10;11)(p13-14;q14-21) encoding MLL-MLLT10 (AF10) [39,40]. About 50% of MLL rearrangements show the t(4;11)(q21;q23) translocation. In general, MLL rearrangements are associated with adverse outcomes [41], due to cellular drug resistance [42]. Neural/Glial antigen 2 (NG2) is a chondroitin sulfate molecule that can be detected by flow cytometry (FC) and its expression was proved to have higher sensitivity and specificity for MLL rearrangement in both ALL and AML [43].

IGH@ translocations: Rearrangements of the immunoglobulin heavy chain locus (IGH@) on chromosome 14q32 are rare in B-ALL, occurring in <5% of cases [44] and it occurs more frequently in adolescents and appears to have poor clinical outcomes [45].

Intrachromosomal amplification of chromosome 21 (iAMP21): is defined as the presence of three or more copies of the RUNX1 gene within a morphologically abnormal chromosome 21 [46, 47]. It occurs in approximately 2% of older children with B-ALL and is associated with poorer outcomes [48].

The prognoses of the most common cytogenetic abnormalities seen in precursor B-ALL cases are collectively shown in (Table 3) [49].

Table (3): Cytogenetic abnormalities and prognoses in precursor B-ALL[49]

Cytogenetics	Genes	Frequency	Prognosis
t(9;22)(q34;q11)	BCR/ABL	25% adults 2-5% children	Poor
t(4;11)(q21;q23)	AF4/MLL	10% adults 5-8% children	Poor
t(1;19)(q23;p13)	E2A/PBX1	5-6% children 3% adults	Poor
t(12;21)(p13;q22)	ETV6(TEL)/AML1	20-25% children 3-4% adults	Favorable
Hyperdiploidy		20-25% children 7% adults	Favorable
Hypodiploidy		1-2%	Poor

Diagnosis of precursor B-ALL:

Diagnosis of B-ALL is based on: morphologic, cytochemical, immunophenotypic, cytogenetic and molecular genetic characteristics [10].

Morphological diagnosis:

Peripheral blood counts and smears examination:

It is usually the first and most important investigation [21]. Most of children with ALL present with anemia which is usually normochromic normocytic in 75% of patients, with a normal to low reticulocyte count. White Blood cells (WBC) counts are either normal or depressed (approximately 50 % of children have WBC counts less than 10,000/µL, while 20% have an initial leukocyte count more than 50,000/µL)[32]. The most important finding is the detection of blasts in stained peripheral blood films, however blasts may be sparse or absent in blood counts less than 2,000/µL [21,50]. Neutropenia (less than 500 neutrophils/µL) is a common phenomenon and is associated with an increased risk of serious infection. Thrombocytopenia with platelet counts less than 50,000/µL is common at diagnosis and approximately half of the cases present with bleeding manifestations such as petechiae and purpura [32].

Bone marrow examination:

The need for bone marrow examination is suggested when atypical cells are seen in the peripheral blood or when there is an unexplained depression of more than one peripheral blood element or if unexplained lymphadenopathy or hepatosplenomegaly is associated with cytopenias [10].

Bone marrow aspiration:

Is the standard method for establishing a diagnosis and provides cells for morphologic, cytochemical, immunophenotypic, cytogenetic and molecular analysis. The diagnosis of ALL is based on the demonstration of lymphoblasts in the bone marrow. By convention, the minimum number of bone marrow lymphoblasts required for diagnosis is set at 20%. In ALL, the marrow is hypercellular with replacement of fat spaces and normal marrow elements by leukemic cells. Megakaryocytes are decreased or absent. Bone marrow lymphoblasts are more homogenous with respect to both morphologic and biologic characteristics than are those in the peripheral blood [10]. They are divided morphologically according to FAB classification in to three subgroups; L1, L2 and L3 [51, 52]. (Figures 2, 3 and 4)

Bone marrow biopsy:

Evaluation of the histology of ALL from biopsy sections becomes important when there are few circulating blasts in the peripheral blood and when the bone marrow is inaspirable due to fibrosis or dense packing of the marrow by lymphoblasts. In typical cases, the marrow cellularity is markedly increased due to infiltration by the densely packed blastic elements with no particular pattern of involvement. On hematoxylin and eosin (H&E) stained sections, the blastic morphology is not easily distinguishable from myeloblasts and the distinction between the "L1" and "L2" blasts recognized in Wright-stained material is also usually not possible. However, Burkitt leukemia does have a particular histologic pattern [53]. (Figure 5)

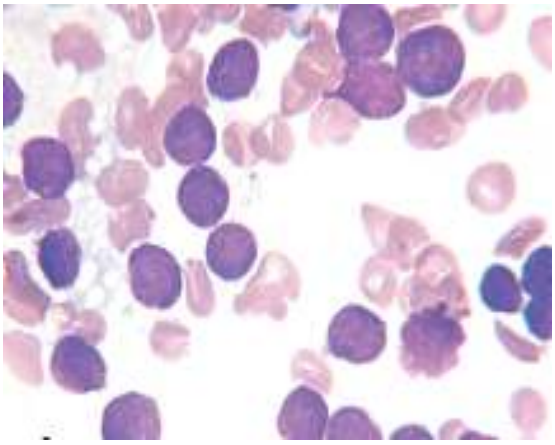


Figure (2): L1 blasts in BMA smear of B-ALL case; (small with scant cytoplasm, fine chromatin and indistinct nucleoli) [52]

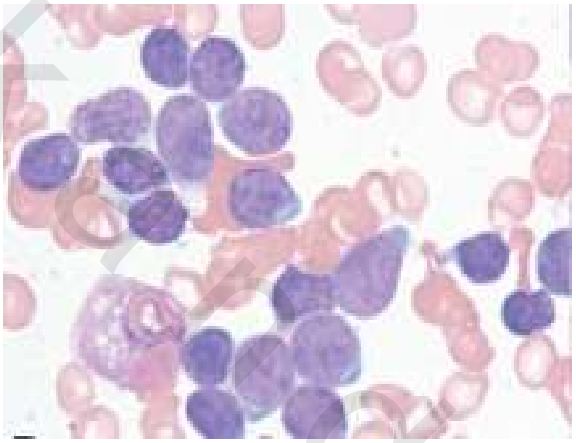


Figure (3): L2 blasts in BMA smear of B-ALL case; (medium to large cells with high nucleocytoplasmic ratio, prominent nucleoli and irregular nuclear membrane outlines) [52]

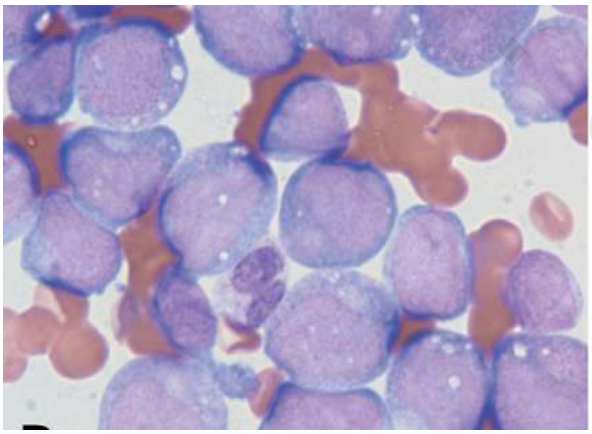


Figure (4): L3 blasts (Burkitt leukemia) in BMA smear ;(medium sized, uniformly rounded nuclei and finely clumped chromatin with diagnostic deeply basophilic and vacuolated cytoplasm) [52]

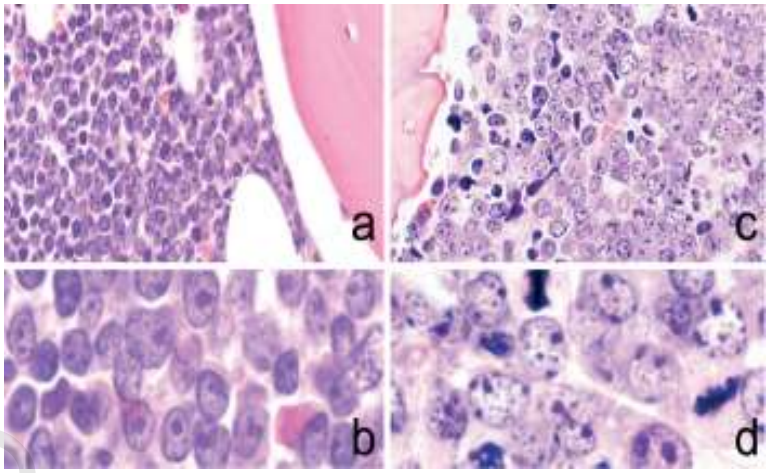


Figure (5): Histologic features of "L1/L2" ALL vs. Burkitt leukemia on H&E stained bone marrow biopsy sections. (a, b) "L1/L2" blasts are equally distributed and have a fine blastic chromatin and variable nucleoli. (c, d) Burkitt leukemia shows a syncytial appearance. The cells are larger, have a punctate chromatin, numerous smaller nucleoli and indistinct cytoplasm. The mitotic rate is markedly elevated [53].

Cytochemistry:

Cytochemistry is used to determine the lineage of the blast cells. The key diagnostic cytochemical feature of ALL is the lack of MPO activity and negativity for non-specific esterase (NSE) [52]. ALL L2 blasts can be easily mixed up morphologically with acute myeloid leukemia blasts (M1 variant) and (M0 variant), so in the first case MPO staining is especially needed to differentiate L2 blasts from (M1 variant), however in the second case (M0 variant); MPO stain is negative (less than 3%), so immunophenotyping is a must to distinguish myeloid leukemia (M0 variant) blasts from ALL [22]. Some cases of ALL exhibit fine SBB positive granules rather than large dark positive granules. Periodic acid-Schiff (PAS) staining is positive in ALL lymphoblasts, showing a large globular pattern. This PAS pattern is not specific and can be seen in erythroleukemia and other leukemia subtypes. Negativity for MPO and NSE should raise the possibility of an ALL diagnosis but further flow cytometric evaluation is necessary as illustrated in (Figure 6) [52].

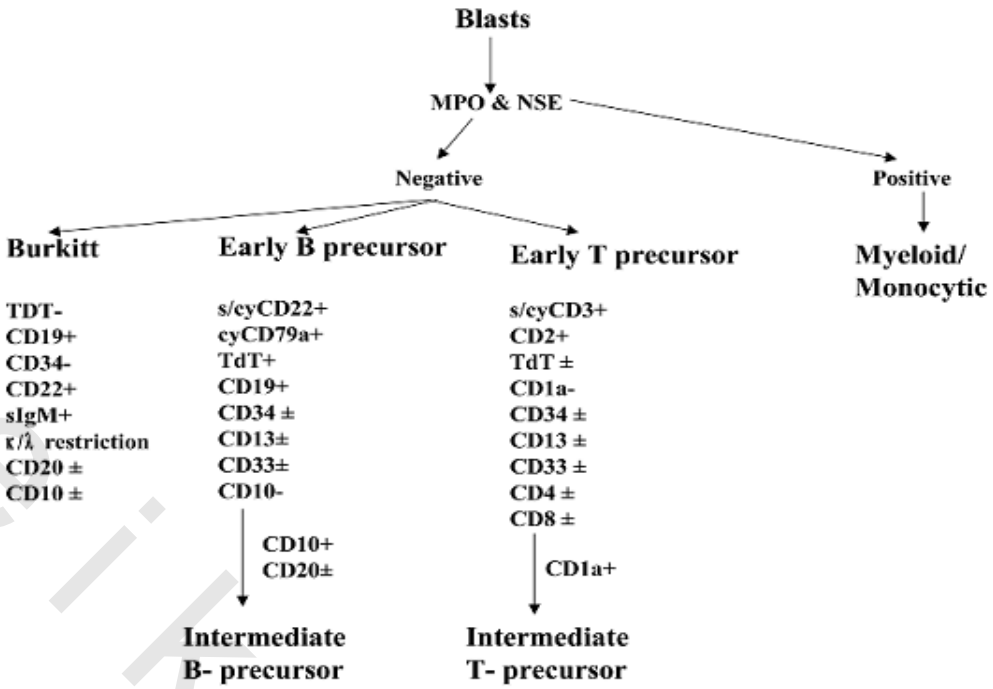


Figure (6): Schematic approach for the diagnosis of blasts that are negative for myeloperoxidase and non-specific esterase [52].

Immunophenotyping:

Immunophenotyping by flow cytometry plays a vital role in the diagnosis, classification, differential diagnosis and detection of minimal residual disease (MRD) in ALL [24]. Precursor B-lymphoblasts express nuclear terminal deoxy-nucleotide transeferase and CD79a. They are weakly positive for CD45 and lack the expression of surface immunoglobulins. Three immunophenotypic categories are identified according to phenotypic expression of certain markers; **pro-B-ALL (pre-pre B-ALL)** which is positive for HLA-DR, TdT, CD22, CD79a and CD19 and negative for CD10[52], **Common B-ALL (CALLA)** which is positive for HLA-DR, TdT, cyt CD22, CD79a, CD19, CD10 and variably positive to CD20[26] and **Pre-B-ALL** which is positive for HLA-DR, cyt CD22, CD79a, CD19, CD10, CD20 and cytoplasmic Ig (mainly IgM) with variable positivity for TdT [27,28]. Burkitt leukemia represented a fourth category named **Mature B-ALL** before being assigned to lymphomas by the WHO. These cells are positive for CD10, CD19, CD20, CD22 and surface Ig. CD34 expression is present in about 40% of B-ALL cases especially in the pro-B-ALL and common B-ALL [49, 54]. (Figure 7)

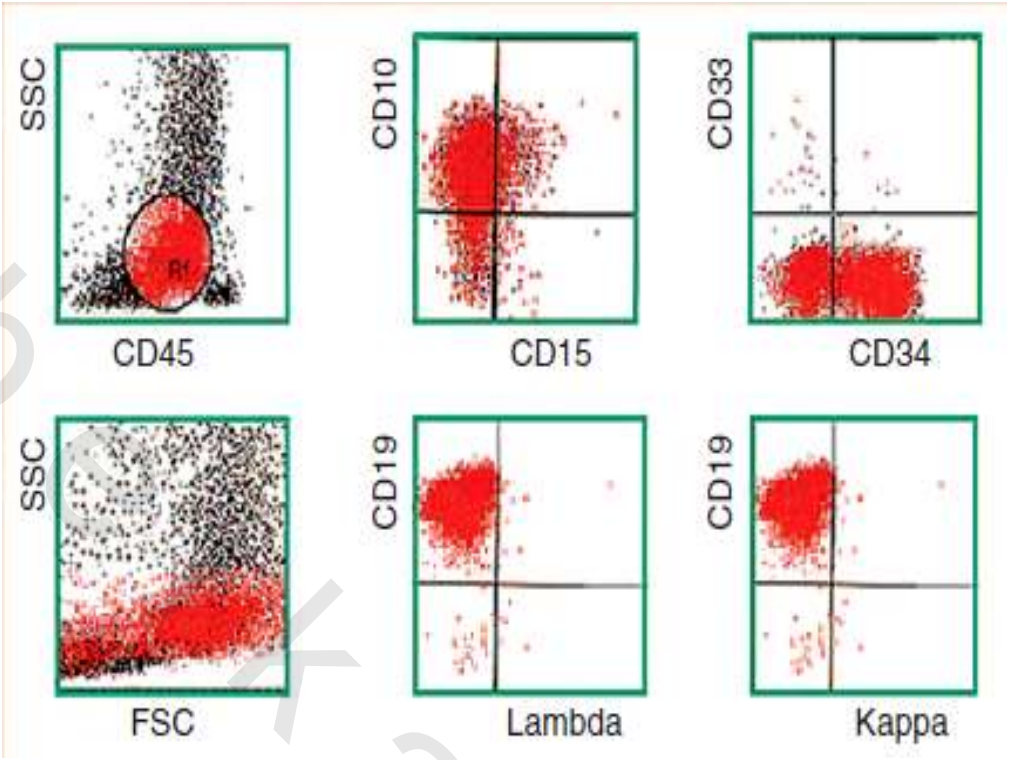
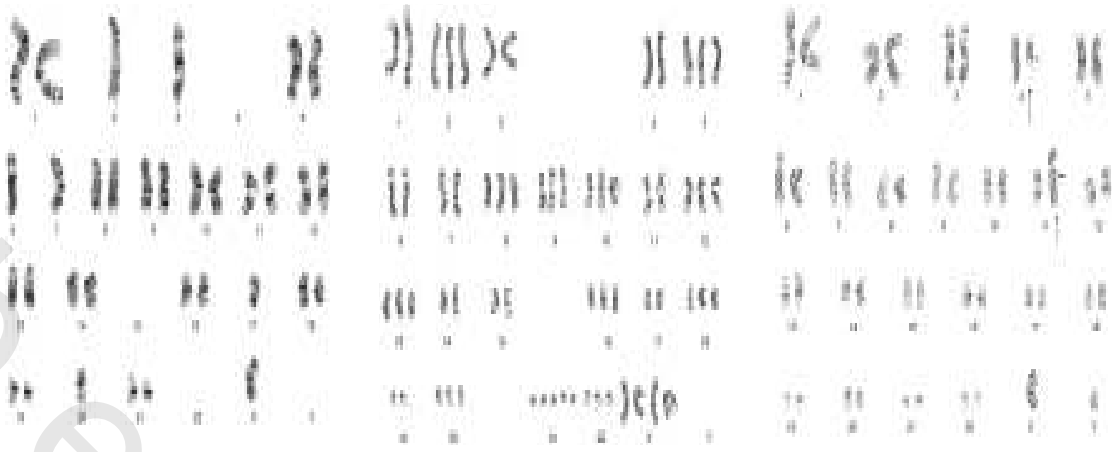


Figure (7): Bone marrow flow cytometric study of a patient with precursor B-acute lymphoblastic leukemia. The blast cells are dimly CD45 positive and express CD10, CD19 and CD34 [(49)].

Cytogenetic and Molecular diagnosis:

Cytogenetic analysis of each patient's ALL cells has become an essential component of diagnosis prior to treatment. It has furthered our understanding of leukemogenesis at a molecular level. Specific and well characterized recurring chromosomal abnormalities facilitate the diagnosis, confirm the subtype and classification and have major prognostic value for treatment planning. Abnormalities in chromosome number or structure are found in approximately 90% of children and 70% of adult ALL patients [(55)]. Approximately 45% of ALL cases demonstrate recurrent ALL specific cytogenetic abnormalities on conventional karyotyping studies (Figure 8) [(52)]. Conventional cytogenetic analysis requires dividing cells which is technically difficult and can be time consuming due to the presence of multiple abnormal cell lines and complex chromosomal banding patterns. Therefore, alternative diagnostic methods have been sought including fluorescence in situ hybridization (FISH), in which labeled probes are hybridized to either metaphase chromosomes or interphase nuclei and then detected with fluorochromes [(55)]. (Figure 9)



(A) **(B)** **(C)**

Figure (8): Conventional cytogenetics of an B-ALL patient showing (A) hypodiploid karyogram, (B) hyperdiploid karyogram and C. t(4;11) [56].

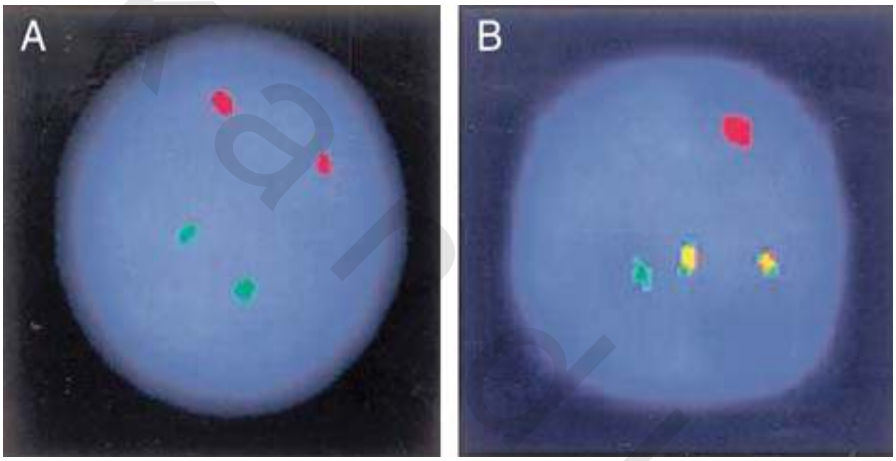


Figure (9): Interphase FISH using dual fusion probes on two separate nuclei to detect; (A) Normal nuclei and (B) nuclei containing BCR/ABL double fusions indicated by green-red fusion (yellow) signals [57].

This method of analysis is more rapid and in some cases more sensitive than conventional cytogenetic analysis. Additionally, FISH can be used to study differentiated or non-dividing cells. In addition, most of these abnormalities can be detected using Southern blotting of genomic DNA and reverse transcription-polymerase chain reaction (RT-PCR) of mRNA, which is a technique in which the fusion mRNA is reverse transcribed into complementary DNA (cDNA) and then amplified by PCR using, gene-specific primers [53]. FISH and RT-PCR are used to detect minimal residual disease and to monitor patients after therapy; real-time RT-PCR allows the quantitative monitoring of residual disease [52].

Cerebrospinal fluid examination:

Lumbar puncture provides an evidence of overt CNS involvement in approximately 3% of children with ALL at diagnosis, most of whom have no neurological symptoms. The evaluation of cytocentrifugation (cytospin) slides made from the cerebrospinal fluid (CSF) enhances the diagnostic sensitivity by concentrating low concentrations of cells and may indicate CNS involvement[10]. The definition of CNS involvement used by the Children's Cancer Group (CCG) is; more than 5 WBC/µL of CSF plus unequivocal blasts identified on the cytospin. However, there is much debate regarding this definition and difficulties in interpretation arise when there are less than 5 WBC/µL of CSF, but blasts are present. One approach has classified CNS leukemia into three groups [53]:

- CNS 1 (< 5 WBC/µL of CSF and no blasts),
- CNS 2 (< 5 WBC/µL of CSF and blasts),
- CNS 3 (> 5 WBC/µL of CSF and blasts or cranial nerve findings).

Flow cytometric immunophenotyping is now considered a useful diagnostic tool for accurate detection of blast cells in the CSF specimens even when the cellularity is low [58].

Biochemical investigations:

Several biochemical changes and electrolyte disturbances can be found in ALL patients. In patients with large leukemic burden; serum uric acid and lactate dehydrogenase (LDH) levels are elevated. If renal impairment is present due to urate nephropathy; serum phosphorous and potassium will be elevated. Elevated liver enzymes; alanine aminotransferase and aspartate aminotransferase (ALT and AST) may be present in 10-20% of the cases. Hypercalcemia may rarely occur due to release of parathyroid hormone like protein from the blast cells [4].

Imaging studies:

Chest radiography can reveal pleural effusion. Skeletal x-ray films may show generalized rarefaction of bones, metaphyseal banding (leukemia lines), cortical osteolytic lesions and periosteal new bone formation (in up to one half of the patients). Ultrasonography may be used to screen for testicular involvement and hepatosplenomegaly. Computerized Tomography (CT) scan of the brain may be needed if intracerebral infiltration or intracranial hemorrhage is suspected [4].

Risk group stratification and prognosis for acute lymphoblastic leukemia in children:

Identification of prognostic factors is critical in determination of therapy for sub-groups of patients to achieve a high cure rate with minimal toxicity. Another important goal of risk stratification is to define patients with a very high risk of relapse, who may benefit from allogeneic hematopoietic stem cell transplantation after the first complete remission. Large study groups defined risk classifications with regards to various risk factors [59]. The most important of these risk classification groups are; National Cancer institute (NCI) risk group

classification, which stratifies risk according to age and WBC count into; standard risk (WBC count less than 50,000/µL and age 1 to younger than 10 years) and high risk (WBC count 50,000/µL or greater and/or age 10 years or older) [60]. The second group is the Children's Oncology Group (COG) risk groups, which stratified children with ALL according to the following prognostic factors; age, WBC count at diagnosis, immunophenotype, cytogenetics/genomic alterations, presence of extramedullary disease, down syndrome and steroid pretreatment. They found that EFS rates exceed 85% in children meeting good-risk criteria (aged 1 to <10 years, WBC count <50,000/µL and precursor B-cell immunophenotype). However, in children meeting high-risk criteria, EFS rates are approximately 75% [61].

However, Berlin-Frankfurt-Münster (BFM) risk groups protocols has been based almost solely on treatment response criteria, which included the following; Standard risk (patients who are MRD negative (i.e. $<10^{-4}$) at both time points), Intermediate risk *(*patients who have positive MRD at week 5 and low MRD ($<10^{-3}$) at week 12) and high risk (patients with high MRD ($\geq 10^{-3}$) at week 12, also patients with a poor response to the prednisone prephase are also considered high risk regardless of subsequent MRD) [62]. The most important prognostic factors for ALL are summarized in (Table 4)[10].

Table (4): Prognostic Factors in Acute Lymphoblastic Leukemia [10]

Determinants	Favorable	Unfavorable
White blood cell counts	$<10 \times 10^9$/L	$>200 \times 10^9$/L
Age	3–7 y	<1 y, >10 y
Gender	Female	Male
Ethnicity	White	Black
Node, liver, spleen enlargement	Absent	Massive
Testicular enlargement	Absent	Present
Central nervous system leukemia	Absent	Overt
FAB morphologic features	L1	L2
Ploidy	Hyperdiploidy	Hypodiploidy <45
Cytogenetic markers	Trisomies 4, 10, and/or 17 t(12;21) (*TEL-AML1*)	t(9;22) (BCR-ABL) t(4;11) (*MLL-AF4*)
Time to remission	<14 d	>28 d
Minimal residual disease after induction	$<10^{-4}$	$>10^{-3}$

Treatment of ALL:

Four main treatment elements can be generally recognized in chemotherapy protocols adopted by international cooperative groups: induction, CNS directed therapy, consolidation/reinduction and maintenance therapy, in addition to Bone Marrow Transplantation (BMT) [32].

Induction therapy: The treatment plan in this phase aims at eradicating signs and symptoms of the disease and re-establishing normal hematopoiesis. This goal is generally indicated with the term of "complete remission" (CR). Children in CR must have no physical evidence of leukemia, normal complete blood cell counts and normally regenerating bone marrow (with < 5% leukemic blasts). Information on CR status also includes the absence of detectable CNS or extramedullary disease, which is evaluated by physical examination and CSF findings [32].

Induction therapy involves administration of vincristine 1.4 mg/m^2 and daunorubicin 45 to 60 µg/m^2 given on days (1, 8, 15 to 22). Prednisone is given twice daily at a dose of 60 mg/m^2 for a total of 28 days and L-asparaginase; 5,000-10,000 units/m^2 added for 7 to 12 days, beginning on day 15 to 17 of induction phase [21]. In addition, a tyrosine kinase inhibitor such as imatinib or dasatinib, should be incorporated into the treatment of patients with t(9;22) (Philadelphia chromosome) positive disease [63-66]. Response to therapy is often assessed by BM examination during the induction phase of treatment. The number and frequency of additional BM examinations performed during induction has not been standardized. Because of this, the definition of "rapid response" and "slow response" vary between institutions. Induction failure, which occurs in fewer than five percent of cases is defined by the persistence of leukemic blasts in the blood, bone marrow or any extramedullary site after four to six weeks of remission induction therapy. This has been considered a particularly ominous sign and is an indication to employ a more aggressive treatment regimen and for allogeneic hematopoietic cell transplantation (HCT) [67].

CNS preventive therapy: The routine use of preventive CNS therapy is a major therapeutic advance in the treatment of childhood ALL. CNS treatment usually begins during the induction phase and continues throughout the remainder of the treatment regimens. Craniospinal radiotherapy was effective in preventing CNS leukemia but was associated with significant toxicity, such as cognitive impairment and altered white matter development[68,69]. As a result, craniospinal radiotherapy has been replaced by intrathecal chemotherapy in several CNS preventive therapy protocols. Intrathecal chemotherapy includes; cytarabine, methotrexate and hydrocortisone [70].

Consolidation therapy: Consolidation or intensification therapy is the second phase of ALL treatment and is initiated soon after attainment of CR. Ongoing treatment is required because small numbers of leukemic lymphoblasts (referred to as minimal residual disease) remains in the BM despite histologic evidence of CR after induction therapy. In such cases, relapse occurs quickly if therapy is not continued. Consolidation therapy usually lasts from four to six months. It commonly involves the use of several different drug combinations and drugs with mechanisms of action that differ from those used during the induction phase. Regimens often include the following drugs {cytarabine, methotrexate, anthracyclines (daunorubicin, doxorubicin), alkylating agents (cyclophosphamide,

ifosfamide) and an epipodophyllotoxin (teniposide, etoposide)}, administered according to a variety of schedules to maximize drug synergy and minimize the development of drug resistance[71,72]. Intensification of therapeutic regimens has been adjusted based upon the patient's risk of poor outcome [73].

Maintenance therapy: The overall treatment duration for most children with ALL is 24 to 36 months. After completion of the consolidation or intensification phase of therapy patients often receive a less intensive continuation regimen using daily oral 6-mercaptopurine (6-MP) [74] and weekly methotrexate with periodic intrathecal therapy[75,76].

Bone Marrow Transplantation: Once patients have had a relapse, irrespective to the duration of the first remission the outcome is always fatal without BMT. Patients who have suffered relapses should be referred for allogeneic transplantation after a second subsequent remission has been attained. If no donors are available autologous BMT or matched unrelated or mismatched related donors may be considered [21].

Minimal residual disease

Minimal residual disease (MRD) is defined as the lowest detectable level of disease in patients in complete clinical remission (with less than 5% blasts in the BM) by the available methods i.e. disease not detectable by morphologic examination and can only be detected by highly sensitive techniques with sensitivity 100 times more than morphological examination, such as multi-parametric flow cytometry (MCF) or polymerase chain reaction (PCR). These techniques have introduced a profoundly new way for monitoring the response to treatment and allowing a more stringent definition of 'remission' in patients with acute leukemia. MRD detection has several important roles such as investigating the presence of tumor cells after therapy, determining efficacy of treatment, monitoring remission status of patients and predicting the possibility of a relapse, as MRD is the major predictor for relapse of leukemia [77-81]. Investigators of the COG reported that the presence of MRD (0.01% or higher) at the end of remission induction (day 29) was the strongest prognostic indicator superior to other commonly used prognostic parameters in childhood ALL and predicted both early and late relapses [14].

The high morphologic threshold for detection of residual disease in patients with precursor B-ALL which is a blast count of 5% of total nucleated cells in BM aspirate smears, results in the inability to measure fluctuations in the leukemia tumor mass with accuracy and relapsed disease can be diagnosed only at its most advanced stages [59].

The most reliable two basic methods for detection of MRD in childhood ALL are; molecular analysis of B- and T-cell receptor gene rearrangements and FC analysis of aberrant immunophenotypes. With both methods, it is possible to detect a single leukemic cell in 10.000 or more normal cells with increased sensitivity of a minimum of 100 folds when compared to conventional light microscopy [82, 83].

Detection of MRD by flow cytometric immunophenotyping:

Flow cytometry is a laser based technology frequently used in science of immunology as well as in hematology, molecular biology, pathology and biology. With monoclonal antibodies, FC has allowed identification and quantification of the major lymphocyte populations and further subdivisions that differ in biologic function,

maturation stage, and activation [59]. One major application of FC is cell immunophenotyping [84], which helps to distinguish between healthy and diseased cells, that can be used to aid the diagnosis and also monitoring of myelomas, lymphomas, leukemias, immunodeficiencies as well as infections [85,86]. FC detection of MRD can be utilized in the majority of cases of both B- and T-lineage ALL and is rapid, relatively sensitive and quantitative with the ability to detect one leukemia cell in a background of 10^3–10^4 normal cells. This is due to the unique property of FC; the ability to distinguish viable cells from BM debris and dead cells which is of vital importance in MRD detection. However, the disadvantages of this technique include a lack of standardization across laboratories with significant variation depending on the expertise of the operator, difficulty in distinguishing between normal regenerating bone marrow progenitors and residual leukemic blasts and the instability of the antigenic expression of the leukemic clone with resultant immunophenotypic shifts during treatment that can result in false-negative MRD results [87, 88].

The challenge in MRD detection is distinguishing residual leukemic cells from non malignant cells especially in conditions where regenerating BM may contain earlier maturation forms than normal samples at a steady stage of hematopoiesis. This presupposes excellent immunophenotypic knowledge of both the malignant clone and the normal BM [89].

For flow cytometric MRD monitoring an aberrant immunophenotype present on the cell surface of the leukemic blasts; known as leukemia associated immunophenotype (LAIP) can be identified at diagnosis and is used for MRD monitoring [87,88].

In B-ALL cases, the detection of LAIP is based on the detection of aberrant expression of a number of antigens on cells homogeneously defined by the combination of CD19, CD34 and CD10. This is mainly important for detecting MRD in patients with common B-ALL (CD10+), in which there is major difficulty in differentiating blast cells from hematogones which can be quite abundant in the regenerating BM [59].

Detection of MRD by Polymerase Chain Reaction techniques

PCR techniques have been used for monitoring of a leukemia specific fusion gene (e.g. BCR-ABL) or a clone specific rearrangement of the immunoglobulin heavy chain (IgH) or T-cell receptor (TCR) genes. PCR amplification of a specific DNA sequence or complementary DNA (cDNA) unique to the leukemia clone can permit identification of one malignant cell among 10^4–10^6 normal cells. PCR based methods are very specific, highly sensitive and widely applicable to the majority of patients with ALL. Nevertheless, if oligoclonal clones are found at the time of diagnosis, it is unclear which clone should be monitored by PCR for early identification of relapse. If using only one oligonucleotide primer the PCR probes can lose specificity for the chosen target region and the clone causing the relapse is possibly not identified. This may lead to false negative results, which is a known problem in childhood ALL [90]. Studies also show that MRD is an important predictor of disease free survival (DFS) after stem cell transplantation (SCT). MRD negative patients prior to transplant have the highest DFS whereas those with high levels of MRD (but in clinical remission) prior to Allogeneic SCT have the lowest DFS, suggesting that the presence of MRD prior to transplant is associated with significantly higher relapse rates following transplant [14,87,88].

Distinction of blast cells from hematogones in precursor B-acute lymphoblastic leukemia

B-lymphocyte progenitor cells, so called hematogones and mature B-lymphocytes are normal bone marrow constituents, which are more prominent in pediatric bone marrows. Although hematogones show a range in overall cell size, these morphologically distinctive cells characteristically exhibit highly condensed, uniform nuclear chromatin and scanty cytoplasm. By using immunophenotyping, B-lineage cells can be divided into three stages of differentiation from very immature cells (CD34 positive, TdT positive) to intermediately differentiated cells (CD10 positive, CD19 positive) to mature B-cells (CD20 positive, sIg positive). Hematogones correspond to the immature and intermediately differentiated B-cell precursors identified immunophenotypically. In some circumstances, the number of hematogones in the BM is increased greatly, especially in children recovering from chemotherapy, other forms of bone marrow injury and non hematopoietic disorders. These cellular populations are especially problematic when identified in the BM specimens of children after therapy for ALL, since hematogones can resemble malignant lymphoblasts by their morphologic features and by expression of an immature B-cell phenotype. Accurate distinction of hematogone-rich lymphoid regeneration from leukemic lymphoblasts is clearly critical for patient's care. There is a need to identify useful immunophenotypic features that most reliably differentiate hematogone-rich lymphoid populations from leukemic lymphoblasts [(91)]. Single and two colors FC do not reliably differentiate hematogones from leukemic lymphoblasts. However, appropriately applied three and four colors multi-parametric flow cytometry are reported to distinguish between these cell populations in nearly all instances. Increased numbers of hematogones may cause problems in diagnosis because of the morphologic features they commonly share with the neoplastic lymphoblasts of ALL (Figure 10) and their common immunophenotypic features with neoplastic B-cell precursor lymphoblasts [(92)].

Leukemic blasts in precursor B-ALL often show aberrant gain or loss of surface antigens or changes in the pattern or intensity of antigen expression, giving rise to an immunophenotypic profile distinct from normal cells. Thus, FC can be used to differentiate leukemic cells from hematogones. However, the immunophenotypic differences between hematogones and leukemic blasts in some cases may be subtle and extensive experience may be required for reliable interpretation of some antigens or for detecting very low level of disease. As a result, the evaluation of MRD in these circumstances can be problematic. The ideal FC marker for detecting MRD in precursor B-ALL should show a consistent pattern of expression in hematogones and an aberrant expression pattern in leukemic cells in a high proportion of cases and there should be minimal overlap in expression between benign and leukemic cells. Moreover, the level of expression in leukemic cells should be stable over time [(93)].

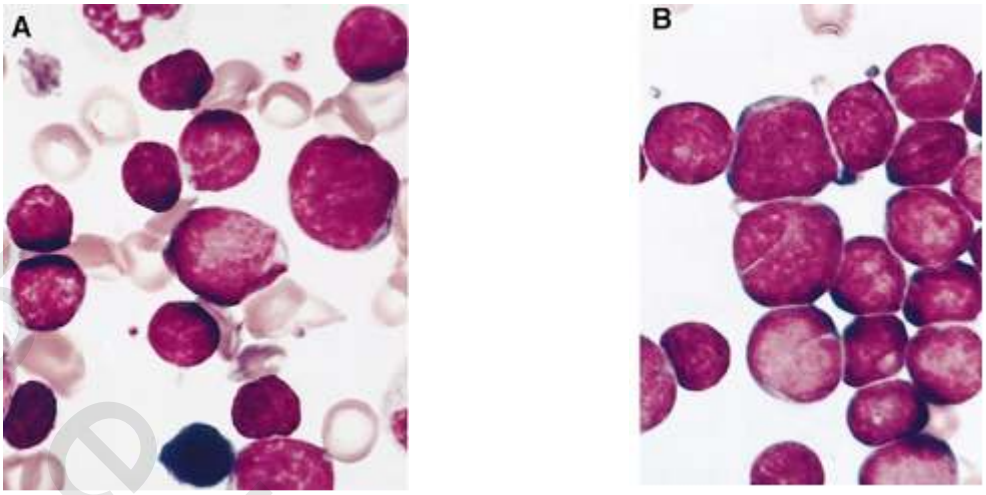


Figure (10): Comparison of the morphology of hematogones and neoplastic lymphoblasts in bone marrow smears.(A) Several hematogones are illustrated in this bone marrow smear from a 3 years old boy with immune thrombocytopenia. They bear close resemblance to the neoplastic lymphoblasts illustrated in panel B. (B) Numerous lymphoblasts are present in the bone marrow smear from a 5 years old girl with precursor B-ALL [92].

CD81 antigen

General features:

CD81, also known as TAPA-1 (target of anti-proliferative antibody) is an integral surface membrane protein that is a member of the tetraspanin family, known to play an important role in multiple cellular interactions by associating with other tetraspanins and partner proteins on the cell membrane[94]. CD81 is associated with CD19 to form a CD19-CD21-CD81multi-molecular complex that is involved in signal transduction in B-cells [95, 96].

The gene locus:

CD81 is encoded by exon 6 on 11p15.5 and its **molecular mass** is 26 kd[10].

Structure:

CD81 is a member of the tetraspanin family, which spans the cell membrane four times. The extracellular region consists of two loops, a small extracellular loop (SEL or EC1) between transmembrane (TM) regions TM1 and TM2 and a large extracellular loop (LEL or EC2) between TM3 and TM4. LEL domain contains four invariant cysteines, two of which define the conserved sequence Cysteine-Cysteine-Glycine. Each LEL is composed of five α-helices (A to E). The first and last form a stalk supporting a mushroom shaped head domain. The head is stabilized by two disulfide bridges. Two hydrophobic patches in the head are indicative of a design for the purpose of protein– protein interactions in keeping with the propensity of CD81 to form multi-molecular complexes with other tetraspanins and integrins. The sequence between TM2 and TM3 is the most conserved region in all tetraspanins, whereas LEL is the most variable [10]. (Figure 11)

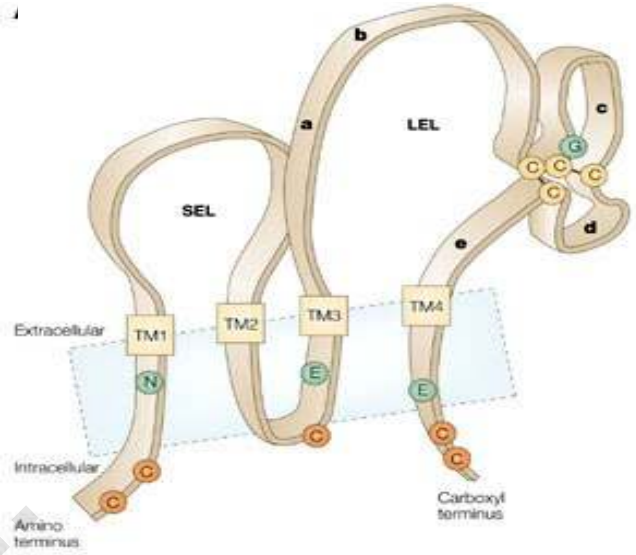


Figure (11): CD81 is shown as the prototypical tetraspanin. The four TM domains contain conserved polar residues (green circles), and they flank the small and large extracellular loops (SEL and LEL, respectively). The LEL is composed of a core formed by helices a, b and e, and this core structure is conserved among the tetraspanins. Helices c and d comprise the variable portion of the LEL, and they are flanked by the CCG motif and further conserved cysteine residues (yellow circles). This region is folded as a result of disulphide bridges (black lines) to form a mushroom-like structure. Potential palmitoylation sites on the intracellular conserved cysteine residues are also indicated (orange circles) [97].

Distribution:

CD81 is detected on most human tissues, except RBCs and platelets [10].

Functions of CD81:

Functions of CD81 in normal physiology have been largely documented for the immune system. On B-cells, CD81 induces surface expression and maturation of CD19. It also forms a complex with CD19 and CD21 [98]. This complex contributes to B-cell activation by bringing together signaling molecules required for B-cell receptor (BCR) signaling [94]. This multi-molecular complex within the CD19, CD21 co-receptor reduces the number of BCRs necessary to trigger B-cell activation [99,100].

Signals delivered through the BCR (the membrane form of Igs) and accessory molecules (CD19/CD21/CD81, CD40 and integrins) are critical for the activation and survival of B-cells. Through an antigen driven selection process in the germinal centre (GC), B-cells undergo apoptosis or differentiate into memory B-cells or long-lived plasma cells that produce high affinity antibodies in serum. Memory B-cells recirculate in the periphery; whereas plasma cells accumulate and survive in the BM. B-lymphocytes express a broad panel of tetraspanin proteins including CD81. In the B-cell membrane, tetraspanins can functionally interact with different 'partner' molecules, including the BCR

complex, major histocompatibility complex (MHC) molecules, β1 integrins, C type lectins and signalling molecules. A crucial step in the humoral antibody response is B-cell activation triggered by the engagement of antigen to the BCR [101]. Figure (12)

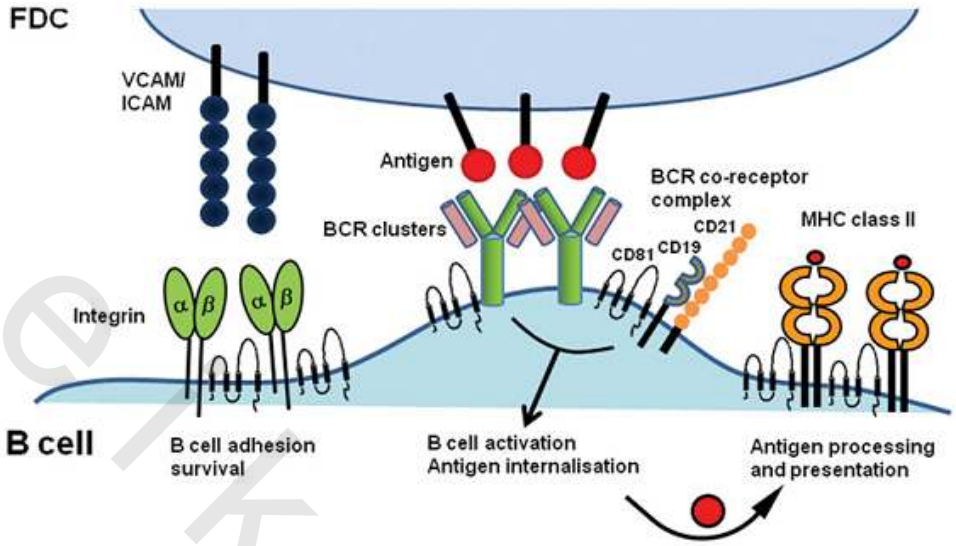


Figure (12): Model of the tetraspanin web in the plasma cell membrane of B-cells during activation [101]

In the GCs of secondary lymphoid organs, follicular dendritic cells (FDCs) present membrane bound antigens to B-cells, which results in the formation of BCR microclusters that is critically regulated by the actin cytoskeleton. This together with the BCR co-receptor complex induces B-cell activation and signal transduction. Moreover, the integrins α1β2 and α4β1 interact with ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) on the FDCs which promotes B-cell adhesion, facilitates antigen recognition and increases the survival. BCR mediated antigen internalization enables antigen processing and presentation on MHC II molecules, which leads to subsequent CD4 positive T-cell activation. Tetraspanins can modulate B-cell activation at three different levels. First, tetraspanin CD81 is a vital component of the BCR co-receptor complex and tetraspanins (CD9, CD53 and CD81) interact with the IgM BCR. Secondly, tetraspanins (CD9, CD81 and CD82) interact with α4β1 integrins and regulate B-cell adhesion. Thirdly, tetraspanins (CD9, CD81 and CD82) may promote or inhibit (CD37) MHC clustering and subsequent antigen presentation [101].

CD81 also plays a role in T-cell receptor signaling and T-cell development in the thymus [92]. An important property of CD81 and of all tetraspanins, is the formation of multi-molecular complexes with other tetraspanins, as well as with integrins in a selective way (e.g.CD81/$\alpha_4\beta_1$, CD51/$\alpha_5\beta_1$, and CD151/$\alpha_6\beta_1$) forming the "tetraspan web", a kind of molecular "organizer". These tetraspanin complexes are facilitators of several functions related to cell adhesion and migration. CD81 and CD151 are responsible for associating these integrins to other tetraspanins, probably through interactions with other tetraspanins. CD81 forms important adhesion complexes with the tetraspanins CD9 and CD151 [10]. In addition, CD81 in complex with CD9 promotes myogenesis; it stimulates myoblast differentiation, fusion and myotube formation and maintenance [102].

Clinical importance of CD81:

The hepatitis C virus is well known to infect human cells by using CD81 as a cell surface receptor for entry into the cell. The hepatitis C viral envelope glycoprotein E2 binds to CD81 and modulates the properties of CD81. In B-lymphocytes, this interaction may help to explain the observed epidemiological associations among hepatitis C infection, lymphoproliferative disorders and non-Hodgkin lymphomas. Binding of E2 to CD81 has been shown to activate naive B-lymphocyte proliferation as well as induce hypermutation of the variable region of immunoglobulin genes in B-cells. Similarly, ligation of CD81 with the co-stimulatory molecule CD28 leads to naive T-cell proliferation, which may contribute to the chronic inflammatory environment seen in hepatitis C infection [103].

CD81 was identified as a potential marker of prognostic significance in patients with diffuse large B-cell lymphoma [104]. This identification was accomplished by statistical analysis of multiple diffuse large B-cell lymphoma gene profiling studies [105-107], which identified CD81 alongside genes; LMO2, MHC class II and BCL6. The potential association of CD81 with LMO2 and other prognostic markers relevant to diffuse large B-cell lymphoma further suggests a role for CD81 in lymphoma pathogenesis [108,109]. Interestingly, CD81 can also be found on both murine and human multi-potent neural progenitor cells and may be clinically useful for detecting circulating neuroblastoma cells [110]. CD81 expression by multi-parametric flow cytometry in multiple myeloma plasma cells was also found to have prognostic impact at diagnosis in symptomatic and high risk smoldering multiple myeloma (SMM) [111].

CD81 expression was reported to be aberrantly dim in a small series of precursor B-ALL cases, which suggested the possible usefulness of CD81 expression in distinguishing precursor B-ALL cells from hematogones [93].