10 Acute Lymphoblastic Leukemia: Clinical Presentation, Diagnosis, and Classification

Sandeep Gurbuxani, Joseph P. Wynne, and Richard A. Larson

10.1 Introduction

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm of lymphocytes characterized by the clonal accumulation of immature blood cells in the bone marrow. These abnormal cells are arrested in the lymphoblast stage of the maturation pathway. Aberrations in proliferation and differentiation of these cells are common, and normal hematopoiesis is suppressed. Symptoms result from varying degrees of anemia, neutropenia, and thrombocytopenia or from infiltration of ALL cells into tissues. Although virtually any organ system may become involved once leukemia cells enter the peripheral blood, the lymph nodes, spleen, liver, central nervous system (CNS), and skin are the most common sites detected clinically.

ALL is a heterogeneous disease with distinct biologic and prognostic groupings. Treatment strategies tailored to specific prognostic groups have already yielded dramatic improvements in the outcomes for children with ALL, and similar risk-adapted strategies based on the biological heterogeneity of the disease are now being applied to adults. Moreover, increasing knowledge of the prognostic significance of recurrent cytogenetic abnormities plays an important role in the current WHO classification of precursor lymphoid neoplasms, and thus, cytogenetics will be emphasized in this review [1].

10.2 Clinical Presentation

10.2.1 Epidemiology

ALL is the most common malignant disease in childhood, peaking in incidence between ages 2 and 5 years [2]. In contrast, ALL only accounts for approximately 20% of acute leukemia in adults. Despite this early peak incidence in childhood, nearly 45% of all new cases are diagnosed in adults (age > 20 years). This is due to a combination of ALL developing in all age groups and a steadily increasing incidence rate above the age of 50 years [3].

The worldwide incidence of ALL is estimated to be 1–5/100,000 [4]. ALL is slightly more common among males than females (1.3:1). Geographic variations with higher incidence rates in Spain and in Latin American countries are likely related to a number of factors including socioeconomics, ethnicity, and an urban or rural setting. A higher frequency of ALL has been reported in industrialized countries and in urban

S. Gurbuxani
Section of Hematopathology, Department of Pathology, University of Chicago and the Comprehensive Cancer Center, Chicago, IL, USA

J. P. Wynne · R. A. Larson (✉)
Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL, USA
e-mail: rlarson@medicine.bsd.uchicago.edu

S. H. Faderl et al. (eds.), *Acute Leukemias*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-53633-6_10

areas. The incidence of ALL is more common in Caucasians compared with African-Americans with an age-adjusted overall incidence in the United States of 1.9/100,000 in whites and 1/100,000 in blacks. Americans of Hispanic ethnicity have the highest incidence rate at 2.5/100,000 compared to 1.6/100,000 for non-Hispanic whites [3].

10.2.2 Presentation

The clinical presentation of ALL is often sudden. Patients commonly present with a recent history of fatigue or spontaneous bleeding. Malaise, lethargy, weight loss, fevers, and night sweats are often present but typically are not severe [5]. Compared to AML, patients with ALL experience more bone and joint pain. Rarely, they may present with asymmetric arthritis, low back pain, diffuse osteopenia, or lytic bone lesions [6]. Children experience these symptoms more frequently than adults with young children often presenting with difficulty walking due to bone pain. Lymphadenopathy, splenomegaly, and hepatomegaly are more common than in AML and affect half of adults with ALL. CNS involvement is also more common in ALL compared to AML [7]. Patients may present with cranial neuropathies, most commonly involving the sixth and seventh cranial nerves. Nausea, vomiting, headache, or papilledema may result from meningeal infiltration and obstruction of the outflow of CSF leading to raised intracranial pressure. Patients may also present with a symptomatic mediastinal mass with symptoms of cough, dyspnea, and/or superior vena cava syndrome if it impinges on the great vessels. Testicular involvement, which presents as a painless unilateral mass, is noted at diagnosis in approximately 2% of boys. It is associated with infant or adolescent age, hyperleukocytosis, splenomegaly, and a mediastinal mass. The diagnosis of testicular involvement can be confirmed by wedge biopsy but is often not needed unless physical examination and imaging studies are equivocal. Bilateral biopsies have previously demonstrated the high incidence of contralateral testicular disease, necessitating radiation treatment to both testicles if testicular involvement persists after induction therapy [8, 9].

The physical examination is often notable for pallor, generalized lymphadenopathy, hepatosplenomegaly, and signs associated with thrombocytopenia, such as gingival bleeding, epistaxis, petechiae/ecchymoses, or fundal hemorrhages. Dermal involvement, known as leukemia cutis, may also be noted.

10.3 Diagnosis

10.3.1 Initial Laboratory Evaluation

The morphologic recognition and phenotypic characterization of lymphoblasts in the blood and bone marrow are of major importance in the correct diagnosis and classification of ALL. These require careful evaluation of well-prepared peripheral blood and bone marrow aspirate smears and phenotypic analysis of the blasts by flow cytometry and immunohistochemistry with an appropriate panel of surface and cytoplasmic markers.

The settings in which lymphoblasts are seen in the peripheral blood and bone marrow aspirate can vary significantly. In the majority of cases, the counts and cellularity are high, but in some, there can be pancytopenia and hypocellularity, which make the recognition of the blasts more critical. A leukoerythroblastic picture can be seen in some cases, and in rare T-cell ALL cases, there may be dysplasia in the granulocytic elements. One unusual morphologic presentation of ALL is that of precursor-B ALL with eosinophilia. This entity can show eosinophilia preceding, concurrent with, or following ALL at either diagnosis or relapse. Sometimes the eosinophilia can be so extreme as to obscure the blasts. This entity is associated with the specific cytogenetic abnormality, t(5;14)(q31;q32) [10]. In other cases, eosinophilia can be indicative of underlying rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM-JAK2* rearrangements. Despite initial presentation as lymphoblastic leukemia, these disorders arise from a pluripotent stem cell and are

classified as myeloid/lymphoid neoplasms with eosinophilia indicating propensity for presentation with many different morphologic features including myeloproliferative neoplasms and acute leukemia [1].

In addition to the peripheral blood and bone marrow, extramedullary involvement with predilection for involvement of the central nervous system (CNS) is common. The evaluation of cytospin slides made from the cerebrospinal fluid (CSF) may indicate CNS involvement. The current approach used to determine CNS involvement by the Children's Oncology Group (COG) has been to classify CNS leukemia into three groups: CNS 1 (<5 WBC/μL of CSF and no blasts), CNS 2 (<5 WBC/μL of CSF with blasts), and CNS 3 (≥5 WBC/μL of CSF with blasts, or cranial nerve findings) [11]. Moreover, some studies advocate using flow cytometry in addition to cytology to identify blasts in the CSF more accurately [12]. Whether this level of detection would affect outcomes is unclear.

In cases with a high cell turnover, the evaluation of blood chemistries may reflect the evidence of tumor cell lysis, such as hypocalcemia, hyperkalemia, hyperphosphatemia, elevated LDH, hyperuricemia, and elevated creatinine.

10.3.2 Cytomorphology

The cytomorphologic characteristics of lymphoblasts are varied, but are usually sufficient to suggest a blastic or neoplastic process for which phenotyping can confirm and further characterize the process. The most typical lymphoblast is a small- to intermediate-sized cell with round or oval nucleus that has a smudgy nuclear chromatin, absent or small nucleoli, and scanty cytoplasm. Comparison to normal appearing "mature" lymphocytes in the blood or marrow aspirate is useful for the assessment of size and degree of chromatin condensation. The scant cytoplasm is quite dramatic in many cells as the nucleus has an appearance of bulging out of the cell cytoplasm. The cytoplasm is pale blue and not intensely stained. In other cases, lymphoblasts exhibit significant morphologic variation. Such lymphoblasts are larger and have oval or irregular nuclear outlines and less homogeneous chromatin. Nuclei are variable but frequently prominent, and sometimes multiple. The cytoplasm is more abundant but still pale blue. The earlier FAB classification into L1 and L2 morphology is poorly reproducible and has little clinical significance. Therefore, the 2008 revision of the WHO classification of ALL adopted a classification based on immunophenotype and genotype [13].

Similarly, while Burkitt lymphoma can have a leukemic presentation, it is now recognized as a malignancy of mature B cells to distinguish it from lymphoblastic leukemia/lymphoma that arises from precursor lymphoid cells. The so-called L3 blasts (referred to as Burkitt leukemia for the remainder of this chapter) are usually quite distinctive. The blasts are large and homogeneous and have distinctive deep blue cytoplasm, which commonly contains sharply defined vacuoles. The nuclei of Burkitt cells are large and round or oval. They have a finely stippled chromatin and variable nucleoli, which sometimes are quite prominent. The larger size and intense cytoplasmic basophilia with vacuolization are decidedly the most distinctive features but are not entirely specific. Vacuoles can be seen in monoblastic and erythroid leukemia, and together with the deep blue cytoplasm, can be seen in other cases of ALL as well as in some cases of AML [14, 15]. Conversely, some cases of Burkitt leukemia with the characteristic chromosomal translocations lack the usual "L3" morphology [16].

A number of additional cytologic variants of lymphoblasts deserve mention. Although there are no particular clinical, phenotypic, or genetic correlates with these variant blasts, their recognition will help avoid exclusion of ALL from diagnostic consideration in cases where they are seen. Small lymphoblasts can be seen in rare cases of ALL [17]. These blasts are closer in size to small "mature" lymphocytes, making them difficult to distinguish from the small lymphoid cells of chronic lymphocytic leukemia (CLL). The small lymphoblasts also have more condensed chromatin making the distinction difficult further. Lymphoblasts with cytoplasmic granulation can be seen in a small percentage of ALL cases

[18, 19]. The granules are usually present in the larger blasts; they are azurophilic and usually not numerous. Nuclear clefts can be seen in some lymphoblasts and are present as deep nuclear groves. The so-called hand mirror cell is probably not a defining characteristic for a distinct entity [20]. Whether such cells are due to an artifact of the preparation is debatable. Different lymphoblasts are illustrated in Fig. 10.1.

10.3.3 Histology

Evaluation of the histology of ALL from biopsy sections becomes important when there are few circulating blasts in the blood and when the bone marrow is inaspirable. It is also critical in evaluating extramedullary sites of involvement such as lymph nodes, testes, or skin. Whether bone marrow biopsies are necessary in the typical patient with a high number of blasts in the circulation and bone marrow aspirate is disputable. However, the biopsy may provide a baseline for cellularity, degree of residual normal hematopoiesis, and the presence of necrosis or other associated features.

In typical cases, the marrow cellularity is markedly increased due to the infiltration by the densely packed blastic elements with no particular pattern of involvement. Rare cases have a predilection for paratrabecular growth, but this is very unusual. On H&E-stained sections, the blastic morphology is not easily distinguishable from myeloblasts. Burkitt leukemia does, however, have a particular histologic pattern. The features are similar to the lymph node involvement by Burkitt lymphoma.

Hypocellular presentations of ALL are relatively rare, but can present a diagnostic challenge due to the paucity of cells and thus limited material for immunophenotyping [21]. Some cases of ALL can present with frank fibrosis [22]. Inability to aspirate could be due to the fibrosis, or in some cases, due to the dense packing of the marrow by lymphoblasts. Necrosis is present in a small number of cases and can complicate the diagnosis, due to the lack of viable cells for either morphologic evaluation or for immunophenotyping [23]. Necrosis can be focal or widespread and can recur with relapsed disease. Occasional cases can show bone changes, which include osteoporosis or osteopenia [24].

In some cases of ALL, the principle manifestation of disease is extramedullary [25]. This is not uncommon in precursor T-cell ALL/lymphoma which can present with a mediastinal mass and lymphadenopathy. Other sites that may be identified prior to blood and bone marrow disease include lymph node, skin, testes, and CNS. Whenever there is concern for a lymphoblastic process in an extramedullary location, careful review of the blood and evaluation of the marrow is imperative.

Differential diagnostic considerations are based on clinical presentation as well as cytomorphologic and histologic features of blasts in the peripheral blood and marrow. Reactive causes of lymphocytosis should be excluded particularly in the pediatric age group where the morphology of the peripheral blasts can be difficult to distinguish from mature lymphocytes. In pediatric patients with high peripheral blood counts, pertussis must be considered. Pertussis can result in lymphocytosis of 20–30,000/μL, and the lymphocytes can sometimes appear atypical, although they should have mature-appearing chromatin. Furthermore, unlike in B-ALL, the hemoglobin and peripheral platelet counts are usually preserved when the lymphocytosis is reactive. In the bone marrow, hematogones or normal B-cell precursors can be increased in number in regenerative situations (Fig. 10.2). These require careful evaluation, as they closely resemble malignant lymphoblasts [26]. Small round blue cell tumors seen in pediatric patients can also mimic ALL in the marrow, but immunohistochemical studies can usually resolve any diagnostic concerns. In adults, leukemic manifestations of mature B-cell lymphoma, particularly the blastic variant of mantle cell lymphoma [27], can mimic ALL. Additionally, high-grade B-cell lymphomas can have a blast-like morphology. Immunophenotyping is needed to resolve the diagnosis in such cases. Mature B-cell lymphomas lack expression of precursor cell markers such as CD34 and terminal deoxynucleotidyl transferase (TdT), whereas they express surface

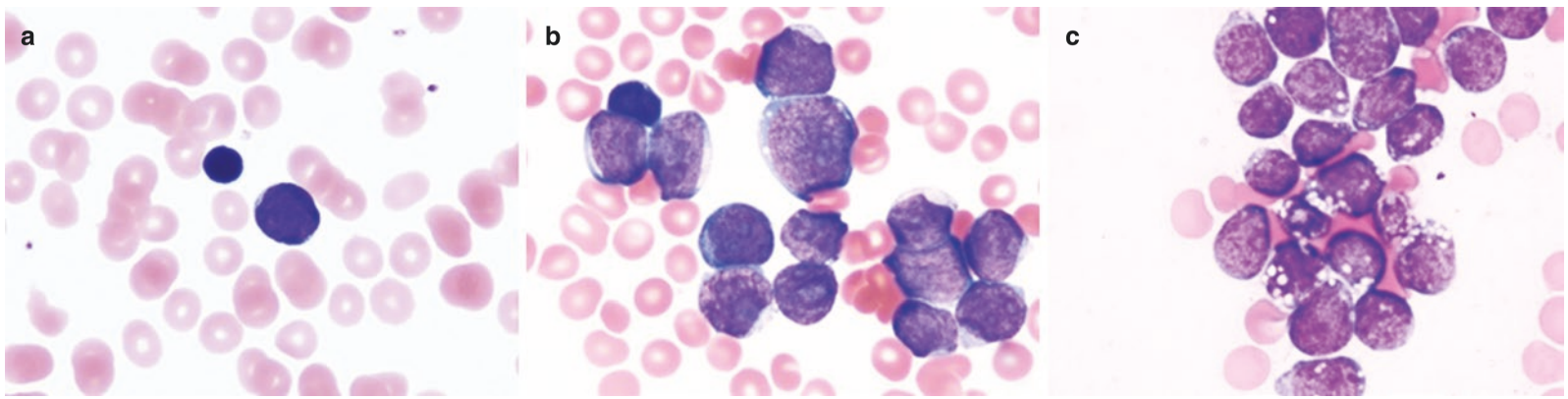

Fig. 10.1 Cytomorphology of lymphoblasts in comparison to Burkitt lymphoma (Wright-stained blood and bone marrow aspirate smears). (**a**) Small uniform blasts are about two times the size of erythrocytes and have a smudgy homogenous chromatin without prominent nucleoli. Comparison to a small lymphocyte (left) is always helpful. (**b**) Varied lymphoblasts, including numerous larger blasts with more open chromatin, prominent nucleoli, and abundant cytoplasm. (**c**) Burkitt lymphoma cells (previously called "L3" blasts) are usually distinctive with homogeneous large size and deep blue cytoplasm with prominent vacuoles. Vacuoles can, however, be seen in some cases of AML and ALL

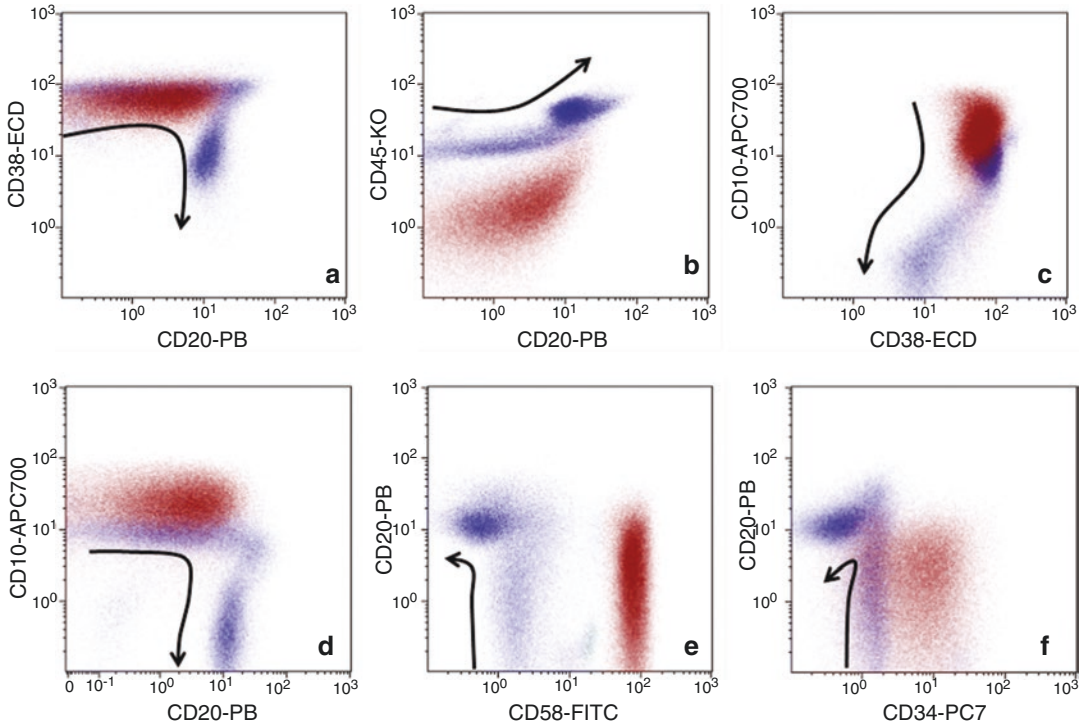

Fig. 10.2 Distinguishing malignant B-lymphoblasts from hematogones by flow cytometry. Hematogones are normal B-cell precursors that can be morphologically difficult to distinguish in regenerative marrow aspirates from blasts. However, by flow cytometry, hematogones follow a distinct and predictable pattern of antigen expression (blue dots and black arrows) (**a**, **c**). In contrast, ALL blasts (red dots) show maturation arrest and differences in intensity of the antigens normally expressed on hematogones. In the example shown here, when compared to hematogones (blue), residual ALL blasts (red) express asynchronous CD20 with weak CD45 (**b**), bright CD10 (**d**), bright CD58 (**e**), and bright CD34 (**f**)

immunoglobulin (sIg) light chains (see discussion on immunophenotyping below). In both children and adults, the differential also includes AML, mixed phenotype leukemia, and chronic myeloid leukemia (CML) presenting in lymphoid blast phase. In all except the latter, immunophenotyping by flow or by immunohistochemistry can resolve the diagnostic dilemma. These differential diagnostic considerations and a list of non-hematologic processes that may resemble ALL are summarized in Table 10.1.

10.3.4 Immunophenotype

Enzyme cytochemistry, particularly the demonstration of reactivity to myeloperoxidase (MPO), can exclude the diagnosis of ALL. Other cytochemical reactions used in the past have been largely replaced by flow cytometry. Multicolor flow cytometry allows for simultaneous detection of multiple antigens on the surface of the leukemic blasts, allowing not simply the correct diagnosis, but also providing a footprint for monitoring for residual disease post-therapy. Flow cytometry can be performed from the bone marrow aspirate or peripheral blood if adequate blasts were present. One should be careful when interpreting the immunophenotype of peripheral blood blasts particularly when the fraction of blasts is low and present with a left shift. The peripheral blood blasts could represent part of the left shift resulting from a leukoerythroblastic phenomenon and may not be representative of bone marrow leukemic blasts. Immunohistochemistry on biopsy sections should

Table 10.1 Differential diagnosis of ALL

Non-hematologic processes:
Tuberculosis
Heavy metals
Human immunodeficiency virus (HIV)
Infectious mononucleosis
Autoimmune diseases
Juvenile rheumatoid arthritis
Osteomyelitis
Hematologic processes: differential diagnostic considerations from cytomorphology and histology
In children
Pertussis
Hematogones
Small round blue cell tumors
In adults
Chronic lymphocytic leukemia (CLL)
Prolymphocytic leukemia (PLL)
Lymphoma, especially blastic variant of mantle cell
Plasmablastic myeloma
Blastic plasmacytoid dendritic cell neoplasm (BPDCN)
In children and adults
Reactive lymphocytosis (mononucleosis)
Thymoma
AML with minimal differentiation (M0) and without maturation (M1)
Mixed phenotype acute leukemia
CML presenting in lymphoid blast phase

Table 10.2 Immunophenotype

(A) Pertinent markers available for immunohistochemical studies
General: TdT, CD34
B-cell markers: CD20, CD79A
T-cell markers: CD3, CD4, CD8, CD5, CD45RO
Myeloid: MPO, CD68, lysozyme, glycophorin A, Factor VIII, CD61
Other: keratin, NSE, myogenin, CD99
(B) Commonly used markers for flow immunophenotyping in acute leukemia
General: CD34, HLA-DR, TdT, CD45
B-cell markers: CD10, CD19, cCD22, CD20, cCD79A, CD24, cytoplasmic μ, sIg
T-cell markers: CD1a, CD2, cCD3, CD4, CD8, CD5, CD7
Myeloid: cMPO, CD117, CD13, CD33, CD11c, CD14, CD15
(C) B-lineage ALL phenotypes
Pro-B: TdT+, CD19/22/79A+, CD10-, cytoplasmic μ−, sIg−
Common precursor-B: TdT+, CD19/22/79A+, CD10+, cytoplasmic μ−, sIg−
Pre-B: TdT+, CD19/22/79A+, CD10+, cytoplasmic μ+, sIg−
(D) T-lineage ALL phenotypes
Pro/immaturethymocyte:TdT+,cCD3+,CD2/5/7+/−
Common thymocyte: TdT+, cCD3+, CD2/5/7+, CD4+/CD8+, CD1a+
Mature thymocyte: TdT+/−, CD3+, CD2/5/7+, CD4+ or CD8+, CD1a−

not be considered as an alternative to multicolor flow cytometry but can be used when a bone marrow aspirate is not available and peripheral blood shows no circulating blasts.

Consensus guidelines for immunophenotyping of leukemia have been proposed by several cooperative groups [28, 29]. While there is no consensus on a single panel, the initial flow cytometry should allow for distinction of leukemic blasts from normal regenerative precursors in the bone marrow or thymus and correctly classify B, T, myeloid, acute leukemias of ambiguous lineage, and blastic plasmacytoid dendritic cell neoplasms. Commonly used markers are listed in Table 10.2B.

The large majority of cases of ALL (~85%) are of B-lineage. B-lineage assignment is based on expression of CD19, CD79a, and CD22. CD45 expression is characteristically weak or absent. In addition, TdT, CD34, and CD10 are variably expressed. Aberrant expression of CD13 and CD33 is common, and in the absence of MPO expression, the expression of these antigens does not preclude a diagnosis of ALL. Combinations of antigens expressed by the leukemic blasts correspond to the degree of maturation and associate with genetic features. The earliest stage of maturation (early precursor-B or pro-B) is characterized by the expression of CD19, cCD79a, and nuclear TdT. This subset is enriched for B-ALL with chromosome 11q23.3 rearrangements. The intermediate stage or common B-ALL is characterized by the coexpression of CD10 along with the above B-cell antigens and is enriched for *BCR-ABL1* and *ETV6-RUNX1* rearranged cases. Finally, the most mature precursor-B cells are characterized by expression of cytoplasmic immunoglobulin

mu chains and enriched for B-ALL with a *TCF3-PBX1* fusion.

While Burkitt lymphoma may have a leukemic presentation, it is a malignancy of mature B cells that express CD19 and CD10. However, in contrast to B-ALL, the malignant cells also express bright CD45, CD20, and monotypic sIg light chains. Rare B-ALL cases can express surface heavy chains (so-called transitional pre-B ALL) while others can show the expression of surface light chains without heavy chains. When seen in the context of appropriate morphology and markers of immaturity such as CD34 and TdT, the expression of surface heavy or light chains does not preclude the diagnosis of precursor B-ALL [30]. B-ALL immunophenotypes are listed in Table 10.2C.

T-ALL accounts for only 15–20% of cases. While CD3 expression is lineage specific, surface CD3 is rarely present. The use of a cytoplasmic tube for flow cytometry to determine the expression of cytoplasmic CD3 is therefore required for definitive diagnosis of T-ALL. Bright surface CD7 expression is invariable but is not T-lineage specific. Other markers that are expressed with variable frequency include CD1a, CD2, CD4, CD5, and CD8. In addition to CD1a, expression of TdT and CD34 are helpful in demonstrating the precursor cell origin of this T-cell malignancy. Similar to B-ALL, a characteristic combination of antigens expressed is indicative of the stage of maturation [31]. These stages are pro-T/T-I, pre--T/T-II, cortical T/T-III, and medullary T/T-IV (Table 10.2D). Expression of myeloid antigens can also be seen in T-ALL. CD13 and CD33 expression has been described in 19–32% of cases [32]. CD117 expression is uncommon and appears to be associated with *FLT3* mutations [33]. It is likely that the CD117-positive T-ALL cases described in the older literature represent mostly what is now recognized as early T-cell precursor ALL (ETP ALL). Initially identified by a distinct gene expression signature [34], this leukemia can be diagnosed based on immunophenotype with the lack of expression of CD1a and CD8, usually negative for CD5, as well as strong positive expression of at least one of CD34, CD117, HLADR, CD13, CD33, CD11b, or CD65. If bright CD5 expression is noted in a CD1a-negative, CD8-negative T-ALL, the diagnosis of ETP ALL can still be made based on bright expression of at least two of the antigens associated with myeloid differentiation (CD13, CD33, CD11b, CD65) or with immaturity (CD34, HLADR, CD117) [29].

Differential diagnostic issues that have to be considered in immunophenotyping include hematogones, thymoma, mixed phenotype leukemia, and CML presenting in lymphoid blast phase. Hematogones have the same immunophenotype as common precursor B-ALL cells, but the hematogones exhibit a spectrum of maturation with a continuum of cells from immature to mature showing loss of CD34 and gain of CD20 and sIg [35, 36].

Thymoma cells have the phenotype of common thymocytes and cannot be distinguished from common T-ALL/lymphoblastic lymphoma by immunophenotype alone. Correlation with clinical presentation and histology is important for the correct interpretation. When CML presents in lymphoid blast crisis, distinction from Philadelphia (Ph) chromosome-positive ALL cannot be made based on immunophenotype, as the blasts are frequently precursor B lymphoblasts. In most Ph+ cases, the presence of a concurrent myeloid component to the leukemia will alert one to the correct diagnosis. If this were not present, lineage analysis showing the BCR/ABL1 fusion in myeloid as well as lymphoid cells has been suggested as a means to differentiate the stem cell process, CML, from the lymphoid-restricted process, ALL [37]. In some cases, only the emergence of a myeloid component after treatment can indicate the correct diagnosis.

10.3.5 Cytogenetic Evaluation

Specific and well-characterized recurring chromosomal abnormalities facilitate diagnosis, confirm subtype 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 [38]. These cyto-

genetic abnormalities are acquired somatic (rather than germline) mutations that frequently result from translocations of chromosomal DNA, resulting in new (abnormal) protein products from the resultant fusion genes. It is assumed that the protein products from these fusion genes are responsible for the cellular dysregulation that leads to the malignant state. Deletions or loss of DNA may eliminate genes that have tumor suppressor functions. Gains of additional chromosomes may lead to gene dosage effects that provide transformed cells with survival advantages.

Conventional cytogenetic analysis requires dividing cells, 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. 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 nondividing cells. In B-ALL, most pediatric and adult patients can be assigned to a genetic-based classification. The most recent revision of the WHO classification recognizes specific B-ALL subtypes with recurrent cytogenetic abnormalities. The inclusion of a given cytogenetic abnormality as a specific entity is based on distinctive clinical or phenotypic properties, prognostic implications, or evidence for biology that is exclusive of other entities. For example, B-ALL with iAMP21 has been added to the entities included in the fourth edition [39]. In addition, *BCR-ABL1*-like ALL has been included as a provisional entity to include a heterogenous group of B-ALL characterized by gene expression profiles indistinguishable from B-ALL with *BCR-ABL1*, but the former lacks the *BCR-ABL1* translocation [40]. B-ALL with this gene expression profile is associated with cryptic translocations involving *CRLF2* or a multitude of tyrosine kinases [41].

10.3.6 Molecular Evaluation

Polymerase chain reaction (PCR) is an enzyme assay that provides a more sensitive and rapid method to detect clonal gene rearrangements. Translocations that result in fusion genes are especially suited for analysis with reverse transcriptase PCR (RT-PCR), a technique in which the fusion mRNA is reverse transcribed into cDNA, and then amplified by PCR using gene-specific primers. Quantitative RT-PCR allows for quantification of measurable (minimal) residual disease (MRD). A number of large prospective studies in pediatric ALL have demonstrated the independent prognostic significance of MRD detection [42, 43]; less is known about the significance of MRD detection in adult ALL [44].

10.4 Conclusion

In summary, the initial approach to the diagnosis of ALL still involves evaluation of the peripheral blood smear and bone marrow specimens with cytomorphology, immunohistochemistry, and cytogenetic analysis. Cytogenetic analysis and molecular methods are used to establish prognostically distinct subgroups of ALL. Through enhanced knowledge of the leukemogenic pathways involved in the different ALL subgroups, one can anticipate improved accuracy in diagnosis and prognosis, and ultimately, improved disease outcomes for these patients.

References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001–2007. Blood. 2012;119(1):34–43.
3. Surveillance, Epidemiology, and End Results (SEER) program populations (1969–2017) [Internet] (2019). National Cancer Institute, DCCPS, Surveillance

Research Program. Available from: www.seer.cancer.gov/popdata. Cited 1 May, 2019
4. Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). Eur J Cancer Care. 2005;14(1):53–62.
5. Clarke RT, Van den Bruel A, Bankhead C, Mitchell CD, Phillips B, Thompson MJ. Clinical presentation of childhood leukaemia: a systematic review and meta-analysis. Arch Dis Child. 2016;101(10):894–901.
6. Gur H, Koren V, Ehrenfeld M, Ben-Bassat I, Sidi Y. Rheumatic manifestations preceding adult acute leukemia: characteristics and implication in course and prognosis. Acta Haematol. 1999;101(1):1–6.
7. Larson RA. Managing CNS disease in adults with acute lymphoblastic leukemia. Leuk Lymphoma. 2018;59(1):3–13.
8. Hijiya N, Liu W, Sandlund JT, Jeha S, Razzouk BI, Ribeiro RC, et al. Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: lack of therapeutic role of local irradiation. Leukemia. 2005;19(8):1399–403.
9. Nachman J, Palmer NF, Sather HN, Bleyer WA, Coccia PF, Lukens JN, et al. Open-wedge testicular biopsy in childhood acute lymphoblastic leukemia after two years of maintenance therapy: diagnostic accuracy and influence on outcome – a report from Children's Cancer Study Group. Blood. 1990;75(5):1051–5.
10. Grimaldi JC, Meeker TC. The t(5;14) chromosomal translocation in a case of acute lymphocytic-leukemia joins the Interleukin-3 gene to the Immunoglobulin heavy-chain gene. Blood. 1989;73(8):2081–5.
11. Winick N, Devidas M, Chen S, Maloney K, Larsen E, Mattano L, et al. Impact of initial CSF findings on outcome among patients with National Cancer Institute standard- and high-risk B-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. J Clin Oncol. 2017;35(22):2527–34.
12. Ranta S, Nilsson F, Harila-Saari A, Saft L, Tani E, Söderhäll S, et al. Detection of central nervous system involvement in childhood acute lymphoblastic leukemia by cytomorphology and flow cytometry of the cerebrospinal fluid. Pediatr Blood Cancer. 2014;62(6):951–6.
13. Jaffe ES. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Geneva: WHO; 2001. p. 351.
14. Al-Rubei K, Rose PE, Hill FG. Morphological heterogeneity in childhood B cell acute lymphoblastic leukaemia. J Clin Pathol. 1984;37(12):1348–52.
15. Lilleyman JS, Hann IM, Stevens RF, Richards SM, Eden OB. Blast cell vacuoles in childhood lymphoblastic leukaemia. Br J Haematol. 1988;70(2):183–6.
16. Khalidi HS, Chang KL, Medeiros LJ, Brynes RK, Slovak ML, Murata-Collins L, et al. Acute lymphoblastic leukemia – survey of immunophenotype, French-American-British classification, frequency of myeloid antigen expression, and karyotypic abnormalities in 210 pediatric and adult cases. Am J Clin Pathol. 1999;111(4):467–76.
17. Kim Y, Kang CS, Lee EJ, Kim WI, Shim SI, Kim SM, et al. Acute lymphoblastic leukemia with maturation – a new entity with clinical significance. Leukemia. 1998;12(6):875–81.
18. Darbyshire PJ, Lilleyman JS. Granular acute lymphoblastic leukaemia of childhood: a morphological phenomenon. J Clin Pathol. 1987;40(3):251–3.
19. Schwarzinger I, Födinger M, Scherrer R, Wolzt M, Mannhalter C, Speiser W. Hypergranular acute lymphoblastic leukemia (ALL). Report of a case and review of the literature. Ann Hematol. 1993;67(6):301–3.
20. Schumacher HR, Desai SN, McClain KL, Domenico DR, Dizikes GJ, Bird ML. Acute lymphoblastic leukemia-hand mirror variant. Analysis for endogenous retroviral antibodies in bone marrow plasma. Am J Clin Pathol. 1989;91(4):410–6.
21. Wegelius R. Preleukaemic states in children. Scand J Haematol Suppl. 1986;45:133–9.
22. Wallis JP, Reid MM. Bone marrow fibrosis in childhood acute lymphoblastic leukaemia. J Clin Pathol. 1989;42(12):1253–4.
23. Macfarlane SD, Tauro GP. Acute lymphocytic leukemia in children presenting with bone marrow necrosis. Am J Hematol. 1986;22(4):341–6.
24. Ribeiro RC, Pui CH, Schell MJ. Vertebral compression fracture as a presenting feature of acute lymphoblastic-leukemia in children. Cancer. 1988;61(3):589–92.
25. Steinherz PG, Siegel SE, Bleyer WA, Kersey J, Chard R, Coccia P, et al. Lymphomatous presentation of childhood acute lymphoblastic-leukemia – a subgroup at high-risk of early treatment failure. Cancer. 1991;68(4):751–8.
26. Longacre TA, Foucar K, Crago S, Chen IM, Griffith B, Dressler L, et al. Hematogones – a multiparameter analysis of bone-marrow precursor cells. Blood. 1989;73(2):543–52.
27. Ott G, Kalla J, Ott MM, Schryen B, Katzenberger T, Müller JG, et al. Blastoid variants of mantle cell lymphoma: frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood. 1997;89(4):1421–9.
28. Bene MC, Nebe T, Bettelheim P, Buldini B, Bumbea H, Kern W, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. Leukemia. 2011;25(4):567–74.
29. Dworzak MN, Buldini B, Gaipa G, Ratei R, Hrusak O, Luria D, et al. AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of pediatric acute lymphoblastic leukemia. Cytometry B Clin Cytom. 2017;94(1):82–93.
30. Vasef MA, Brynes RK, Murata-Collins JL, Arber DA, Medeiros LJ. Surface immunoglobulin light chain-positive acute lymphoblastic leukemia of FAB L1 or L2 type – a report of 6 cases in adults. Am J Clin Pathol. 1998;110(2):143–9.

31. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
32. Uckun FM, Sather HN, Gaynon PS, Arthur DC, Trigg ME, Tubergen DG, et al. Clinical features and treatment outcome of children with myeloid antigen positive acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 1997;90(1):28–35.
33. Hoehn D, Medeiros LJ, Chen SS, Tian T, Jorgensen JL, Ahmed Y, et al. CD117 expression is a sensitive but nonspecific predictor of *FLT3* mutation in T acute lymphoblastic leukemia and T/myeloid acute leukemia. Am J Clin Pathol. 2012;137(2):213–9.
34. Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10(2):147–56.
35. Rimsza LM, Larson RS, Winter SS, Foucar K, Chong YY, Garner KW, et al. Benign hematogone-rich lymphoid proliferations can be distinguished from B-lineage acute lymphoblastic leukemia by integration of morphology, immunophenotype, adhesion molecule expression, and architectural features. Am J Clin Pathol. 2000;114(1):66–75.
36. van Wering ER, Van der Linden-Schrever B, Szczepanski T, Willemse MJ, Baars EA, Van Wijngaarde-Schmitz HM, et al. Regenerating normal B-cell precursors during and after treatment of acute lymphoblastic leukaemia: implications for monitoring of minimal residual disease. Br J Haematol. 2000;110(1):139–46.
37. Anastasi J, Feng J, Dickstein JI, Le Beau MM, Rubin CM, Larson RA, et al. Lineage involvement by BCR/ABL in Ph+ lymphoblastic leukemias: chronic myelogenous leukemia presenting in lymphoid blast vs Ph+ acute lymphoblastic leukemia. Leukemia. 1996;10(5):795–802.
38. Le Beau MM, Larson RA. Cytogenetics and neoplasia. In: Hoffman R, Benz Jr EJ, Shattil SJ, Furie B, Cohen HJ, editors. Hematology: basic principles and practice. 3rd ed. New York: Churchill Livingstone; 2000. p. 848–69.
39. Swerdlow SH et al (eds) (2017) WHO classification of tumours of haematopoietic and lymphoid tissues. revised 4th edn. International Agency for Research on Cancer, Lyon, 585p
40. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10(2):125–34.
41. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22(2):153–66.
42. Cavé H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. N Engl J Med. 1998;339(9):591–8.
43. van Dongen JJ, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet. 1998;352(9142):1731–8.
44. Stock W, Luger SM, Advani AS, Yin J, Harvey RC, Mullighan CG, et al. A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. Blood. 2019;133(14):1548–59.