CLINICAL DIAGNOSIS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN LYMPHOBLAST CELL CULTURE: A LITERATURE REVIEW

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Introduction

Acute lymphoblastic leukemia (ALL) is an aggressive heterogeneous disease characterized by the expansion of lymphoid blasts in blood, bone marrow, and other organs [4]. It represents approximately 80% of childhood leukemias and remains the most common malignancy in children [7]. The diagnosis of ALL is established by the presence of lymphoblasts or stem cells predominating in the bone marrow, and childhood leukemia is considered lymphocytic unless cells are characterized by unequivocal Auer rods and/or definite features of myelocytic, monocytic, or histiocytic differentiation [7]. Contemporary diagnostic approaches integrate cell morphology, immunophenotyping, cytogenetics, molecular genetics, and—increasingly—lymphoblast cell culture studies, which provide valuable insights into disease biology and therapeutic targets.

Morphological Assessment

The morphological classification of lymphoblasts has been standardized by the French-American-British (FAB) cooperative group [7]. This classification consists of three subtypes: L-1 (most common) with small cell predominance, scanty cytoplasm, and dense homogeneous chromatin; L-2 with a heterogeneous cell population, the majority being large cells having greater amounts of cytoplasm; and L-3 with an enlarged, uniform, basophilic cell population with round nuclei, dense granular chromatin, and one or more prominent nucleoli [7]. There are indications that the prognosis is worse with types 3 and 2, in decreasing order, and better in L-1 cases [7].

Romanowsky-based stains such as Wright-Giemsa and Giemsa provide the greatest cytoplasmic detail for evaluation of cytomorphology [0]. The diagnosis of ALL generally requires demonstration of ≥20% bone marrow lymphoblasts upon hematopathology review of bone marrow aspirate and biopsy materials, which includes morphologic assessment of Wright-Giemsa stained bone marrow aspirate smears and H&E stained core biopsy and clot sections [0]. According to WHO criteria, for the diagnosis of ALL, the determining factor is infiltration of the bone marrow by blasts in a count greater than 25%, which arbitrarily distinguishes ALL from lymphoblastic lymphoma [6]. The main task of morphological examination of bone marrow aspirate is to determine the relative proportion of lymphoblasts among nuclear cells of hematopoiesis, to distinguish acute myeloid leukemia with minimal myeloid

differentiation (AML M0 by FAB classification), and Burkitt-type leukemia, which has a specific morphological picture (L3 blasts) [6].

Cytochemical Analysis

Childhood ALL blast cells are universally negative to peroxidase, Sudan black, and naphthol AS-D chloroacetate esterase (NCE) stains, but the periodic acid-Schiff (PAS) reaction is positive in at least a few blast cells in most cases [7]. The characteristic reaction is that of coarse granules or blocks in the cytoplasm; the reaction may be so heavy that blocks of strong reactivity have been noted in the cytoplasm [7]. Acid phosphatase reaction was noted by Catovsky to be associated with blast cells of ALL patients with T markers on their leukemic cells [7]. These cytochemical stains are essential for distinguishing ALL from acute myeloid leukemia and other hematological malignancies.

Immunophenotyping

The cornerstone of ALL diagnosis is immunophenotyping, because it is antigen expression that confirms the B-cell or T-cell precursor nature of the neoplastic cell [9]. When evaluated by flow cytometry, the immunophenotype of precursor B-cell ALL typically includes the following surface antigens: CD10, CD19, CD20 (may be very weak or absent), CD22, CD24, CD34, CD45 (weaker than normal lymphocytes), and HLA-DR [9]. The central role in ALL diagnosis is played by immunophenotyping using flow cytometry; this method promptly determines whether blasts belong to the B- or T-lymphoid lineage and simultaneously allows determination of the degree of differentiation [1].

The evaluation of ALL by flow cytometry is fundamental because it allows: (1) distinguishing B-ALL from T-ALL (in addition to identifying rarer forms such as undifferentiated acute leukemias or mixed phenotype leukemias); (2) sending samples to reference laboratories for Ph-like predictor testing in all B-ALL cases; (3) monitoring minimal residual disease (MRD); (4) evaluating positivity for specific antigens (CD20, CD22, and CD19), for which targeted therapies exist (rituximab, inotuzumab, and blinatumomab); and (5) stratifying into prognostic subgroups (e.g., CD1a negativity in T-ALL) [5]. The diagnostic accuracy of immunophenotypic classification is very high, with identification of a lymphoid phenotype [9].

Cytogenetic and Molecular Diagnostics

Cytogenetic analysis is an essential component of the diagnostic workup for ALL. Cytogenetic analyses on cells derived from CD34+/CD10- and/or CD34+/CD19- subfractions from patients at weeks 4 and 6 revealed that 58% to 100% of cultured cells had the same karyotypic abnormality as the patient at diagnosis [2]. Conventional cytogenetics on at least 20 mitoses according to ELN indications and eventual FISH for identification of translocations, monosomies, and chromosomal deletions in metaphase

blasts are recommended [5]. Most cases of ALL have recurring cytogenetic and/or molecular abnormalities that contribute to the phenotype [10].

The molecular classification of B-cell acute lymphoblastic leukemia (B-ALL) has been significantly advanced by gene expression profiling data, leading to an expansion of entities in the recent International Consensus Classification (ICC) and 2022 WHO Classification of Tumours [10]. Molecular biology for the detection of recurrent fusions and molecular genetics are essential for comprehensive diagnosis [5]. In patients with confirmed ALL diagnosis, in the absence of other molecular markers, it is recommended to send bone marrow blood samples to accredited laboratories for identification of patient-specific molecular probes, useful for evaluation of minimal residual disease (MRD) at the end of the third cycle of therapy [5].

Lymphoblast Cell Culture in ALL Diagnosis

Lymphoblast cell culture represents a valuable tool in ALL diagnosis and research. Lymphoblasts derived from the blood of patients are preferred for certain diagnostic applications because lymphoblasts are faster growing and easier to culture than fibroblasts, and they are immortalized cells [11]. Same-day, overnight, and two-day cultures are recommended for ALL and most B cell lymphoma cases, and 72-hour cultures for T-cell lymphomas [11]. Bone marrow from lymphoid leukemias is generally cultured overnight with short exposure to colcemid before harvesting [11]. Lymphoid cells are generally less amenable to culture than myeloid cells [11].

In-vitro culture systems have been used to selectively grow malignant cells from the bone marrow of patients with acute T-lymphoblastic leukemia, and this culture system is also useful for detecting occult malignant cells in histologically normal bone marrow [13]. Bone marrow-derived stromal cells prevent apoptotic cell death in B-lineage ALL: when B-lineage ALL cells are placed in culture, signs of programmed cell death become rapidly apparent in most cases, but stromal support maintains viability [13].

The establishment of continuous ALL cell lines has contributed significantly to the understanding of leukemia biology. A new cell line (NALL-1) was established in vitro from the peripheral blood of a patient with ALL, and chromosome analysis revealed a hypodiploid karyotype [2]. ALL cell lines established at present have covered most of the immunophenotyping and specificity chromosome abnormalities, which promote the progress of genetics diagnosis and target therapy [14]. The HXEX-ALL1 cell line, established from a childhood ALL patient, grew in suspension as single cells or clumps and resembled the morphology of lymphoblastic leukemia cells in the patient's bone marrow smear at diagnosis and second relapse [3]. These cell lines serve as invaluable tools in many immunology and leukemia studies, and guidelines on their proper usage have been established [14].

Clinical Diagnostic Workup

The diagnostic algorithm for suspected ALL begins with first-level assessments including complete blood count with peripheral smear (MGG) for morphological examination, immunophenotype on peripheral blood, complete chemistry and coagulation with D-dimer and ATIII, blood group determination, ECG and chest X-ray, and cultures if fever is present [5]. When morphological and immunophenotypic findings on peripheral blood have confirmed the diagnosis with lineage indication (myeloid or lymphoid) within 8 hours, second-level assessments should be performed as soon as possible with bone marrow aspirate for morphological examination with MGG staining and immunophenotype with flow cytometry [5].

Suspicion of ALL is expressed based on pathological findings in the blood count, especially in the differential count of leukocytes, where lymphoblasts are typically present along with varying degrees of neutropenia, anemia, and thrombocytopenia [6]. For definitive diagnosis, it is necessary to perform detailed and immediate examination of bone marrow aspirate with a panel of highly specialized methods including morphological, immunophenotypic, cytogenetic, and molecular genetic examination [6]. It is recommended that diagnostic bone marrow aspiration be performed in a specialized hemato-oncology center that has the capability to examine the obtained aspirate comprehensively and completely [6]. Bone marrow collection for diagnostic examinations must be performed immediately after suspicion of ALL is expressed and before the initiation of specific antileukemic treatment, including corticosteroids [6].

Conclusion

The clinical diagnosis of acute lymphoblastic leukemia in the context of lymphoblast cell culture represents a multidimensional approach that integrates morphological assessment, cytochemical analysis, immunophenotyping, cytogenetic and molecular diagnostics, and in-vitro culture studies. The Bethesda System and WHO classification provide standardized frameworks for reporting and risk stratification. Lymphoblast cell culture serves both as a diagnostic adjunct—enabling karyotypic analysis, detection of occult malignant cells, and establishment of continuous cell lines for research—and as a platform for understanding the biology of ALL and developing targeted therapies. Future advances in molecular diagnostics and culture techniques are expected to further enhance diagnostic precision and therapeutic options in ALL.

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