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Case Report

The Better View on Acute Lymphoblastic Leukemia Including Peripheral Blood and Bone Marrow Cells as Well as Hematopoietic Microenvironment

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ABSTRACT

Adult Hematopoietic Stem Cells (HSCs) are undifferentiated cells capable of self-renewal and remain component in the process of hematopoiesis which together with the cells that makeup the bone marrow stromal environment and other factors. However, when this process of cell production is unbalanced; leading to an exacerbated and uncontrolled proliferation of blood progenitor cells, so leukemia may develop. We know, the basis of acute leukemia classification remains morphology and cytochemistry. The examination of both peripheral blood and bone marrow films are necessary for the diagnosis and classification of ALL. After the slides study, we can say that leukemia is the consequence of stepwise some alterations that confer both proliferative and survival advantage, as well as self-renewal capacity to the malignant cells including precursor cells, blast cells and other differentiated immature cells as well. In ALL, lymphoid precursor cells are arrested in the early stages of development by the abnormal expression of genes. As a result, RT-PCR or FISH analysis is required to detect this rearrangement.

Keywords: Hematopoietic stem cells; RT-PCR; Progenitor cells

INTRODUCTION

In lab studies, blood cells differential counts are usually the first and most important investigations. White Blood Cell (WBC) is variable which can be between (0.1-1500) × 109/L. The most important finding is the presence of blasts in peripheral blood film. Blasts may be sparse or absent in blood film, however a careful peripheral blood smear examination in well stained slide is very important. Deep neutropenia and thrombocytopenia is usual. In general, Bone Marrow (BM) aspiration confirms the diagnosis of ALL. Aspirate slides should be stained for Romanowsky and cytochemical stains. It has been suggested that Sudan Black B (SBB) reaction is less specific than Myeloperoxidase (MPO) at cytochemical stain because positive reactions have been reported in ALL. The Periodic Acid-Schiff (PAS) reaction is useful for supporting the diagnosis of ALL. PAS-block positivity, which is characteristic of ALL, correlates with the immunological phenotype, being more common in B lineage cases. Also, the (12;21) is the most common genetic lesion in childhood ALL and it is not easily detected by cytogenetic analysis because of the similarity and banding patterns of 12p and 21q [1-3].

CASE STUDY

ALL and its classification

ALL is a malignant clonal proliferation of lymphoid progenitor cells and immature cells in the bone marrow. These abnormal cells are arrested in the lymphoblast cell of normal maturation process. In ALL, cases have a clonal abnormality mostly that can be detected by cytogenetic or molecular analysis. Now, molecular analyses have been widely used in practice to support the clinical criteria which have been used [1-2].

In ALL initial laboratory diagnosis, the morphological of lymphoblasts in the bone marrow and peripheral blood smears, are important basically to the correct diagnosis and classification of ALL. In this regard, the FAB morphologic classification was introduced in 1976 by Bennett and coworkers. According to morphology, most of childhood leukemia are classified as ALL and the others as Acute Myeloid Leukemia (AML). ALL is classified to L1, L2 and L3 [3-6].

Significant of blast morphology: In ALL, the most typical lymphoblast is small or medium sized cell with round nucleus with a nucleoli possibly. This lymphoblast is less likely to be confused

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with myelobasts. Blasts in cases of burkitt lymphoma/leukemia are large and homogenous and have distinct deep blue cytoplasm which possibly with sharply defined vacuoles [7]. The cells nuclei are large and round or oval with stippled chromatin and variable nucleoli. Cases with the morphologically varied lymphoblast were referred to as ALL-L2 by the FAB. Small lymphoblasts can be seen in ALL rare cases. These cells are closer to small mature lymphocytes which making the distinction further difficult. Anyhow, the L1/L2 morphology prognostic significance is not clear. A correlation between L2 morphology and a slow early response to treatment has also been described [8-11]. We can say, the role of morphology as an independent prognostic factor has not been confirmed by others (Figure 1). In lab studies, blood counts with differential are usually the first and most important investigations. White cell count is highly variable falls between (0.1-1500) × 109/L. The most important finding is the presence of blasts in peripheral blood smear. Blasts may be sparse or absent in blood film, however a careful peripheral blood smear examination in well stained slide is very important [4-6,12]. Profound neutropenia, and thrombocytopenia is usual. Bone Marrow (BM) aspiration usually confirms the diagnosis of ALL. Aspirate slides should be stained for Romanowsky and cytochemical stains. As we Know, the basis of the classification of acute leukemia remains on morphology and cytochemistry. The examination of both peripheral blood and bone marrow smears are necessary for the diagnosis and ALL classification [1-2,6-7,13-15,18,26].

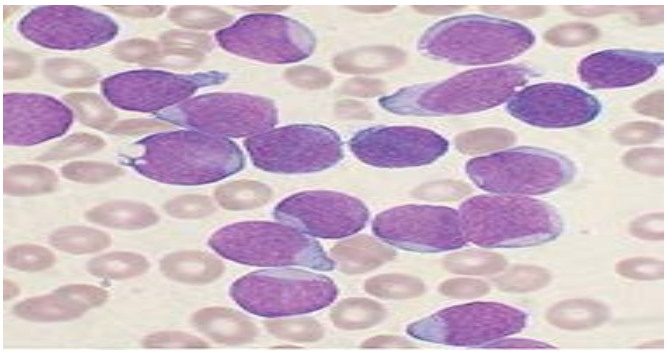

Figure 1: Lymphoblast in peripheral blood smear.

In this case, lymphoblast exhibit significant morphological variation. Such nuclear outlines and less homogeneous chromatin. Nuclei are variable but frequently prominent, and sometimes multiple. The cytoplasm is more abundant but still pale blue. These cases with this more variable lymphoblast usually contain at least some typical lymphoblast. Also, the lymphoblast can confuse with myoblasts likely. This case with the morphologically varied lymphoblast refer as ALL-L2 [18,21,26].

Cell morphology characteristics

The characteristics of lymphoblasts are varied but can sufficient to suggest a blastic or neoplastic process generally 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 the lymphocytes in the blood or marrow aspirate are useful for the assessment of size and degree of chromatin condensation. The cells are larger than small lymphocytes (usually twice the size), and the chromatin is more homogeneous and less condensed and the shape of a block. The scant cytoplasm can be 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. Lymphoblasts with these typical features have been considered as lymphoblasts in L1 subtype according to the French-American-British (FAB) classification and are particularly common in cases of pediatric [1,6-8,18,26].

In some cases, lymphoblasts exhibit significant morphological variation. Such nuclear outlines are less homogeneous chromatin. Nuclei are variable but frequently prominent, and sometimes multiple. The cytoplasm is more abundant but still pale blue (figure 1). Cases with these more variable lymphoblasts contain at least some typical lymphoblasts usually, which are helpful to note, as they are less to be confused with myeloblasts. Cases with the morphologically varied lymphoblasts were referred to as ALL-L2 by in FAB classification, but this classification is now believed to have little significance and the terminology is used here only for descriptive purposes. Some researchers stated that in L1 and L2 subtypes of ALL do not define specific disease entities, show no consistent correlation with phenotypic or cytogenetic features, and have not been adopted in the WHO classification of ALL, which is based on immunophenotype and genotype [3-6].

Compared to the blasts described above, blasts in cases of Burkitt lymphoma/leukemia 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 is the most distinctive features 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. Conversely, some cases of Burkitt leukemia with the associated chromosomal translocations lack the usual L3 morphology [4,6].

Small lymphoblasts can be seen in ALL rarely. These blasts are closer in size to small mature lymphocytes, making the distinction further difficult. Lymphoblasts with cytoplasmic granulation can be seen in the ALL small percentage. The granules are usually present in the larger blasts rather than in the small type of L1. They are azurophilic and not numerous generally. They are usually readily distinguished from granules seem in myoblast but are not distinguishable from the granules seen in natural killer cells. Nuclear clefts can be seen in lymphoblast. Also, they are usually present as deep nuclear grooves and are unlike the clefts seen in circulating follicular lymphoma cells; also hand-mirror cell is probably not a defining characteristic for a certain entity. Whether such cells are due to an artifact of the preparation is debatable [1,6-10].

Thus, acute leukemias of lymphoid cells have been subdivided based on morphologic characteristics by the French-American-British (FAB) group. According to the classification, lymphoid malignancies of small uniform blasts were called ALL- L1, cases with the morphologically varied lymphoblasts were referred to as ALL-L2. These cells exhibit less homogeneous chromatin, variable nuclei but frequently prominent and sometimes multiple and also lymphoid malignancies of uniform cells with basophilic and sometimes vacuolated cytoplasm were called L3. The nuclei of these

cells (Burkitt cells) are large and round or oval and variable nucleoli which sometimes are quite prominent. They have a finely stippled chromatin. Conversely, some cases of ALL-L3 with the associated chromosomal translocation lack the usual L3 morphology [4,8-10].

Differential diagnosis

On H&E stained sections, sometimes, the blastic morphology is not easily distinguishable from each other supposedly, between myeloblasts , and the L2 blasts which recognized in Wright-stained material is also some occasions not possible. Burkitt leukemia does, however, have a particular histological pattern. The features to the lymph node involvement by Burkitt lymphoma. There is an extensive and diffuse infiltrate, a syncytial appearance to the mass of cells, a starry-sky background, and monotonous cells with round, intermediate sized nuclei with moderately condensed chromatin and multiple small nucleoli. Hypo-cellular presentations of ALL are relatively rare but can present a diagnostic challenge due to the paucity of cells and limited material for immunophenotyping. Some cases can present with frank fibrosis, but increased reticulin is more common. Some cases can be fibrosis or to the dense packing of the marrow by lymphoblasts. Necrosis is present in a few number of cases and can complicate the diagnosis due to the lack of viable calls for either morphological evaluation or for immune-phenotyping [1-2,4-9].

Differential diagnostic considerations based on the cytomorphological and histological features of blasts in the peripheral blood and marrow depend in part on the patient's age. In pediatric patients with high peripheral blood counts, pertussis must be considered. Pertussis can result in lymphocytosis of 20/μl-30000/μl, and the lymphocytes can sometimes appear atypical, although they should have mature-appearing chromatin. In the bone marrow, normal immature lymphoid elements can be increased in number in regenerative situations. These require careful evaluation as they closely resemble malignant lymphoblasts. Evaluation of the clinical history and carful interpretation of flow immunophenotyping is critical to rule out ALL (see below). 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, Chronic Lymphocytic Leukemia (CLL) and leukemic phase of lymphoma, particularly the blastic variant of mantle-cell lymphoma, can mimic ALL. Although these can usually be distinguished by morphological features, it is not uncommon to see an initial misinterpretation due to poorly prepared peripheral blood smears. Immunophenotyping is needed to resolve the diagnosis in such cases. A blastic type of plasma cell myeloma in the marrow might also provide some initial diagnostic in adults, but this should be resolved with immunohistochemistry for kappa and lambda. In children and adults, the differential also includes Acute Myeloid Leukemia (AML), bi-phenotypic 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 [6-7,11-15].

Phenotypic evaluation

It begins with cytochemical studies, and specifically with a myeloperoxidase or Sudan black B reaction, as well as non-specific esterase reactions (ANA, ANB) to quickly exclude most cases of AML. AML with minimal differentiation (FAB:M0), erythro-leukemia and megakaryoblastic leukemia are the exceptions as these require, additional studies for exclusion. Additional cytochemistry, such as PAS and oil red, are being used less commonly due to the reliance on surface and cytoplasmic markers evaluated by flow cytometry and immunohistochemistry. Thus, the most useful cytochemical stains are either Sudan Black B (SBB) or Myeloperoxidase (MPO). It has been suggested that SBB is less specific than MPO because positive reactions have been reported in ALL. The Periodic Acid-Schiff (PAS) reaction is useful for supporting the diagnosis of ALL. PAS-block positivity, which is characteristic of ALL, correlates with the immunological phenotype, being more common in B- lineage cases [1,6,17].

In addition, immunophenotypic analysis is critical to confirm a morphological diagnosis of ALL, to resolve a difficult diagnosis and to further sub-classify cases into precursor-B and precursor-T lineage types. However, a specific immunophenotype identified at diagnosis might also be useful for evaluating residual disease by flow cytometry. Most immunophenotyping studies are performed from blood or marrow aspirates with surface and cytoplasmic by flow cytometry, but a growing number of markers are now available for immunophenotyping on tissue sections by immune-histochemical techniques. This is of importance especially in cases which have low peripheral blood blast counts and in which bone marrow material is insufficient for flow analysis or in which a diagnosis is being made from an extra-medullary site. Currently, there are many markers available for tissue immunophenotyping in acute leukemia. There are various recommendations concerning which antibodies to include in a routine flow cytometric panel for the work-up of an acute leukemia, but there is no uniformly accepted panel [1-2,5-6,14-16].

CONCLUSION

After the PB and BM study, we can say clearly, Leukemia Stem Cells (LSCs) possess several key properties of normal cells including self -renewal, unlimited proliferative potential, infrequent or slow replication. LSCs infiltrate the bone marrow and interfere with the normal Hematopoietic Stem Cell (HSC) microenvironment hemostasis. Thus, the major difference between leukemia growth and normal tissue renewal is that whereas normal transit amplifying cells usually differentiate and die, in various levels of differentiation and maturation cells, the leukemia transit-amplifying cells fail to differentiate normally and instead, accumulate, resulting in leukemia growth. Actually, this is a malignant or tumor microenvironment. In other words the relationship between malignant microenvironment and stem-ness is very closer or in fact, stem-ness as a guiding principle that governs and maintains the stem cell state. Thus in the majority of hematopoietic malignant diseases, stem-ness is in the center of these changes at BM microenvironment which can be destroyed in partial or complete after maximally tolerance [19-25]. Regarding HSCs are expelled from the microenvironment (niche) by LSCs and so resulting LSC microenvironment supports leukemogenesis or LSCs not only fill and occupy the HSC environment but also after the remodeling, an autonomous strengthening malignant environment happen (ruined), and possibly leukemia reach to a peak.

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