Routine Immunophenotyping in Acute Leukemia, the Importance of Lineage Assessment

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We present the case of an adult male patient, where the assessment of cell line could not be done without corroboration of the immunophenotype and cytological analysis. The correct lineage assessment is needed in order to treat the patient correctly. Morphology, cytochemistry, and immunophenotyping were used and the diagnosis we established was B acute lymphoblastic leukemia with aberrant myeloid markers (CD13, CD33). Periodic Schiff Acid stain was very useful to obtain an accurate diagnosis. Adult B acute lymphoblastic leukemia usually has an unfavorable prognosis because of certain cytogenetic abnormalities (Philadelphia chromosome) and different reactivity to treatment. This case strongly supports the continued use of immunophenotyping in the diagnosis and monitoring of acute leukemia and corroboration of different diagnostic techniques for the diagnosis.

Keywords: ALL, immunophenotyping, cytochemistry

Introduction

We present a case of acute leukemia, where immunophenotyping and other specific diagnostic techniques were used together in order to obtain an accurate diagnosis. The diagnosis of acute leukemia is not always easy because lineage assessment may be difficult. The correct assessment of lineage is needed for correct treatment. Myeloid and lymphoid lineage can be determined based on morphology, cytochemistry, and immunophenotyping. The case we are about to present is eloquent for this purpose.

Case presentation

Our case is a 66 year-old male patient, who was admitted to the Hematology Department of Medical Clinic I, Tîrgu Mureș, Romania, at the beginning of 2011.

The paraclinical investigations used were:

- Complete blood count (CBC);
- Biochemical investigations;
- Serology tests;
- Bacteriological investigations;
- Blood smear;
- Bone marrow (BM) smear;
- Cytochemical staining: peroxidase staining (MPO), Periodic Acid Schiff (PAS);
- Immunophenotyping:
  - We used a BM sample and blast selection was done using low expression of CD45. Surface antigen expression was considered positive if at least 20% of blasts showed positive labeling;
  - The technique was lyses-wash;
  - The following panel of antibodies was used:
    - CD3/CD19/CD45;
    - CD10/CD19/CD45;
    - CD20/CD2/CD45;
    - CD34/HLA-DR/CD45;
    - CD7/CD5/CD45;
    - CD34/CD1a/CD45;
    - CD34/CD13/CD45;
    - CD34/CD33/CD45;
    - CD15/CD117/CD45;
    - CD56/CD11c/CD45;
    - CD34/CD36/CD45.

Results

Routine CBC showed pancytopenia, with low number of leukocytes (1840/μL), low blood hemoglobin level (8.83 g/dl) and low number of platelets (137,000/μL). The erythrocyte sedimentation rate was 131 mm/h. No signs of infection were present (negative blood cultures for both aerobic and anaerobic bacteria, negative serological tests for Cytomegalovirus, Hepatitis virus and Human Immunodeficiency Virus). On these bases the suspicion of acute leukemia raised.

A blood smear was performed but there was no evidence of blast cells in the peripheral blood. We then performed the morphological examination of the bone marrow and it showed 86% blast cells with polymorphic appearance.

Cytochemical staining for peroxidase was negative, but the PAS staining was positive.

Immunophenotyping results came out positively for the following markers:

- CD19 = 95%;
- CD10 = 55%;
- CD34 = 92%;
- HLA-DR = 92%;

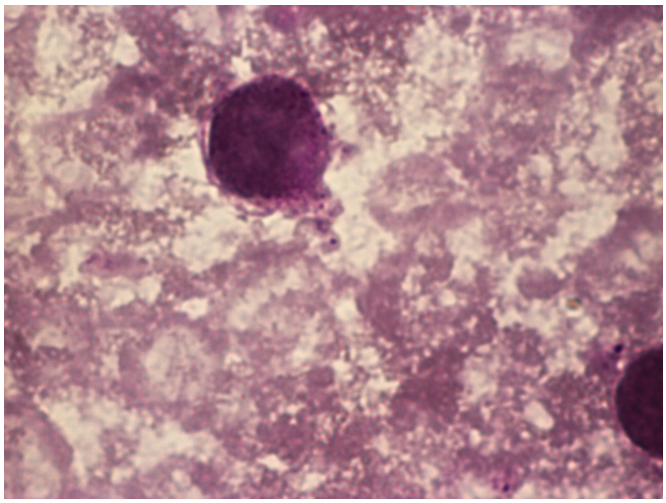

Fig. 1. PAS positive lymphoblast

- CD13 = 92%;
- CD33 = 92%.

So, both lymphoid and myeloid markers were positive. Based on cytochemical staining results (PAS positive) and immunophenotyping the established diagnosis was B acute lymphoblastic leukemia (ALL) with aberrant myeloid markers. The patient started the treatment for ALL.

Discussions

Lineage assignment is essential for optimal treatment in acute leukemia, because treatment regimens are different in ALL and AML.

The question in this case was if the diagnosis is acute leukemia with mixed phenotype, acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). Based on the recommendations of the Word Health Organization 2008 [1], we excluded the diagnosis of mixed phenotype acute leukemia because of the negativity of MPO cytochemical staining, a necessary marker to prove commitment to myeloid lineage. Rarely, cytochemical MPO reaction may be positive in a small number of blasts with clear lymphoblastic morphology [2]. The existence of mixed phenotype acute leukemia (MPAL) is in concordance with the hypothesis of a common progenitor. In this manner also the cases with B + myeloid markers and T + myeloid markers are concordant with the existence of a common B, T, granulocytic-macrophage progenitor [3,4].

PAS staining by itself is 52% sensitive and 81% specific for lymphoid lineage and it is 100% specific when other cytochemical staining are negative (in this case MPO) [5,6]. This finding supports our diagnosis of ALL.

The differential diagnosis with hematogones (abundant in the BM of healthy infants and children and also in cytopenias of different etiologies) was not necessary because our patient was an adult [7].

Myeloid markers such as CD13 and CD33 are frequently expressed in ALL. Different studies report different percentages of myeloid marker in ALL. One study reported CD13 positive (54%), CD 33 positive (43%) of ALL cases. The same study showed little difference between the frequency of myeloid markers in leukemic lymphoblasts in children and adults [7]. Another study showed 27% of lymphoblast positive for myeloid markers [8]. So, a wide expression of myeloid markers was reported on different studies.

In adult ALL, unlike childhood ALL, the number of long complete remission (CR) and survivors was not significantly improved.

Some groups tried to make an age adapted therapy in adult ALL, but without significant results in terms of overall survival (OS) [10].

There is still controversy regarding the prognostic significance of myeloid markers in ALL. Some studies suggest that there is no significant association between the expression of myeloid antigens and CR, and also no association was found with gender, age or clinical manifestations [9]. Eventually a slower response to treatment was reported, but without difference regarding CR or OS achievement [11].

Philadelphia chromosome represents the most common cytogenetic finding in ALL (in 15–30% of cases and the incidence is increasing with age). In both adults and children prognosis of Ph+ ALL is unfavorable. The expression of myeloid markers was more frequently reported in Ph+ ALL than in Ph– ALL. This suggests a poor outcome in ALL with myeloid markers [12]. The CR was significantly lower in patients with Ph+ ALL than in patients with Ph– ALL.

The expression of CD34 is common for Philadelphia positive patients (Ph+). CD10 is more commonly negative in adults, but is positive in our case. Literature reports CD10 positive in Ph+ B-ALL, CD34 positive and bright CD10 expression in B-ALL with t(12;21) and CD34 positive in B-ALL with hyperploidy [7]. Unfortunately we could not perform cytogenetics in this case.

Conclusions

This case supports the utility of both cytological and immunophenotypical investigation of acute leukemia. Lineage assessment is an important goal for correct therapy and the particularities of cases strongly support the continued use of immunophenotyping in diagnosis and monitoring of acute leukemia.

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