Acute Leukemia of Ambiguous Lineage, Biphenotype, without CD34, TdT or TCR-rearrangement

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Abstract

Biphenotypic acute leukemia (BAL) is a rare entity that comprises 0.5-3% of all acute leukemias and probably arises from multipotent progenitor cells. The optimal approach for BAL therapy is unknown. Thus, it is important to elucidate the origin of the neoplastic cells for determination of the appropriate therapy. We report the case of a 41-year-old man with BAL having myeloid and T-lymphoid lineage phenotypes. Strangely, neither CD34 nor TdT expression nor rearrangement of TCR-α/β, δ/γ genes were shown. This pattern is rarely encountered and suggests that the blast cells were possibly considered immature with aspects of differentiation indicating myeloid lineage, rather than T-lymphoid lineage.

Key words: biphenotypic acute leukemia, TCR-rearrangement, TdT, CD34

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Introduction

Acute leukemias (ALs) are broadly classified as having a myeloid or T/B-lymphoid lineage based on the expression of surface or cytoplasmic antigens of proliferating blasts, as well as on morphological features. In addition to these classes, there is a rare type of acute leukemia that is difficult to classify, and which concurrently has morphologic, cytochemical and immunophenotypic characteristics of both myeloid and T- or B-lymphoid lineages. The recent WHO classification refers to this type of entity as bilineage AL, or biphenotypic AL (BAL), and places it into a subtype of the group ‘acute leukemia of ambiguous lineage’ (1, 2).

Bilineage AL is defined by the detection of two separate blast populations, with each population expressing markers of a distinct lineage. In contrast, BAL is characterized by one blast population that co-expresses B-lymphoid and myeloid markers, or, less frequently, T-lymphoid and myeloid markers. The relationship between bilineage AL and BAL is not completely clear (1, 2). The scoring system for markers proposed by the European Group for the Immunologic Classification of Leukemia (EGIL) is useful for diagnosis (3), but its performance against objective benchmarks, or in establishing their origin and lineage, is not clear.

Acute leukemias of ambiguous lineage, including BAL, constitute about 0.5-3% of all acute leukemias (3-5). The most common immunophenotype, representing 60-65% of all BAL, is co-expression of myeloid and B-lymphoid markers, while 25-30% of BAL cases show co-expression of myeloid and T-lymphoid markers, and the trilineage phenotype is rare (3, 4, 6). Several reports have listed the most frequent type of BAL, based on FAB classification, as L1, L2 or M2, whereas the M4 and M5 types are seen only rarely (3, 4, 7). It is generally believed that BAL is derived from multipotent stem cells in bone marrow that can express antigens from more than one cell line (4). Therefore, most cases of BAL show expression of early hemopoietic stem cell markers such as CD34 (3, 4).

Since knowledge about BAL is limited, it was not possible to deduce with certainty its origin or etiology in our patient. It is also unclear, both in terms of clinical and biological presentation and also with regard to treatment outcome,
whether patients with BAL should be treated with regimens designed for acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL) or both (4, 6). Herein, we report a rare case of BAL with myeloid and T-lymphoid lineage phenotypes, which did not show expression of CD34 antigen or TdT and showed no TCR-α/β or δ/γ rearrangement.

Case Report

A 41-year-old man with general fatigue, sore throat and fever was admitted to the nearest hospital. He did not have a significant past medical history. His symptoms did not improve, and many abnormal, atypical lymphocytes were found on laboratory examination of his peripheral blood. The patient was transferred to our hospital for evaluation of suspected acute leukemia in April.

Physical examination revealed a body temperature of 36.2°C, blood pressure of 125/70 mmHg, a heart rate of 60/min, and a body weight of 67.2 kg. He was conscious and alert, and pale conjunctiva was not found. No bleeding tendency was observed, and there was no lymphadenopathy or hepatosplenomegaly. Laboratory examination revealed a white blood cell count of 7,000/μL (neutrophil 0%, eosinophil 1.5%, basophil 0%, monocyte 6%, lymphocyte 26.5%, blast cell 66%), a hemoglobin of 13.1 g/dL, platelet count of 14.8×10⁴/μL, a slightly elevated serum lactate dehydrogenase (LDH) level of 275 IU/L, a C-reactive protein (CRP) concentration of 0.38 mg/dL, albumin concentration of 3.9 g/dL, and serum or urinary lysozyme concentration of 11.9 μg/mL [4.2 - 11.5] and 0 mg/mL/day [<1.1], respectively. Examination of bone marrow aspirate showed a hypocellular marrow consisting of more than 70% of blast cells. There were no dysplastic changes in myeloid, erythroid, or megakaryocytic lineage cells. There was a mixture of blast cells of various sizes resembling monoblasts that had only a few granules, plenty of cytoplasm, fine vacuoles in the cytoplasm, oval or baculiform nuclei with smooth or rough chromatin, and a large prominent nucleolus (Figs. 1A, 1B). However, the blast cells showed negative results for myeloperoxidase (MPO) (Fig. 1C) and nonspecific esterase (α-naphthyl butyrate esterase) reactions (Fig. 1D). Conventional cytogenetic analysis showed a normal karyotype (46, XY). Fluorescence-activated cell sorter (FACS) analyses revealed the immunophenotypic features of the blast cells: they stained positive for CD33, CD65, CD117, MPO (Fig. 2A), CD2, CD3 (cytoplasmic; cyt), CD7 (Fig. 2B), and HLA-DR antigens. Certain B-lymphoid markers (CD79a, CD19, CD20 and IgM) were negative (Fig. 2C). Although the CD3 cyt positive rate in the blast population seemed weak, the re-evaluation comparing the CD3 cyt positive population to negative control, revealed that 20.2% of blast cells definitely expressed CD3 cyt (Fig. 2D).

Based on those assessments, and using the EGIL system, we finally arrived at a diagnosis of acute leukemia of ambiguous lineage, biphenotypic acute leukemia (BAL) that co-expressed myeloid and T-lymphoid lineage antigens with monocytic features morphologically, according to the WHO classification. The blast cells had poor characteristics of T-lymphoid cells, in which molecular studies did not show rearrangement of the immunoglobulin heavy chain and TCR-α/β, TCR-δ/γ genes. Moreover, they were uncommonly
Figure 2. Antigen expression profile of bone marrow blasts. Analysis was performed on a FACS-can flow cytometer, gating on the blast population; all samples contained more than 60% of blasts. Double labeling was carried out with FITC (fluorescein) and PE (phycoerythrin) conjugated monoclonal antibodies. Nuclear and cytoplasmic staining was carried out by flow cytometry after fixation and permeabilization of the cells. A) The myeloid associated antigens CD33, CD65, CD117, and MPO were positive. CD34 was negative. B) T-lymphoid associated markers CD2, CD3 (CD3cyt) and CD7 were expressed. TdT and CD34 were not expressed. C) The expression of certain B-lymphoid markers (CD79a, CD19, CD20 and IgM) are negative. D) 20.2% of blast cells expressed CD3cyt.
negative for terminal deoxynucleotidyl transferase (TdT) and for CD34 antigen (Figs. 2A, 2B).

To treat BAL, the patient received a conventional induction chemotherapy regimen designed for AML [cytarabine (Ara-C: 100 mg/m\(^2\)/day at 1-7 days), idarubicin (IDR: 12 mg/m\(^2\)/day at 1-3 days)]. He responded well to this treatment, which resulted in a complete remission (CR).

Discussion

We report an uncommon case of BAL in which the blasts had monocytic features. The blasts also co-expressed antigens associated with myeloid lineage (CD33, CD65, CD117, and MPO) and T-lymphoid derivation (CD2, CD3cyt, and CD7). Acute leukemias demonstrating immunophenotypic features of more than one cell lineage are referred to as acute leukemia of ambiguous lineage type in the recent WHO classification. A scoring system for markers proposed by the EGIL is useful to diagnose such cases (3, 8, 9). Biphenotypic leukemia is diagnosed when the score is 2 or more for the myeloid lineages and 2 or more for one of the T-lymphoid lineages (8). According to this system, our patient’s score was 5 for the myeloid lineage and 3.5 for the lymphoid lineages.

At this time, there are no generally accepted treatment protocols for patients with BAL. Rubio et al (10), in a series of seven cases of BAL with T-lymphoid and myeloid markers (T-BAL) who received a chemotherapy designed for ALL consisting of cyclophosphamide, vincristine, prednisone and either daunorubicin or idarubicin as induction therapy, reported that 5 of 7 patients could not achieve CR, but all of them were in CR after salvage therapy (mitoxantrone plus high-dose Ara-C) as used in AML (10). On the other hand, Aribi et al reported that 80% of T-BAL patients treated as the first therapy with regimens devised for treating ALL (HCVAD [high-dose cyclophosphamide, vincristine, Adriamycin and dexamethasone]) entered CR (6).

We therefore emphasize that in order to choose the appropriate therapy and better understand the biological basis of BAL, it will be necessary to elucidate the nature or origin of the neoplastic cells. In the present case, FACS analysis showed positivity of the blast cells for MPO antigen. Expression of the myeloid markers CD33 and CD13 is reported to correlate with peroxidase activity (11), suggesting that expression of MPO-PE is related to CD33 expression. Although the blasts were also revealed to be CD3cyt/CD7-negative and CD8-negative, the expression of stem cell marker CD34, earlier lymphoid marker TdT, and TCR-α/β or δ/γ gene rearrangement was not shown. In addition to these findings, the expression of common myeloid progenitor cell marker CD117 related to stem cell factor (SCF) receptor was positive, while CD13 associated with monocytes was negative, possibly suggesting that these blast cells were immature cells differentiating to the myeloid lineage, rather than to the T-lymphoid lineage. Another reason the blast cells were considered immature with aspects of differentia-

tion indicating the myeloid lineage, rather than the T-lymphoid lineage, was their lack of expression of stem cell marker CD34 expression, of the earlier lymphoid markers TdT and TCR-α/β, and of δ/γ gene rearrangement in this case. Expression of these markers is usually characteristic of CD3cyt/CD7-positive lymphoid blasts.

It is believed that BALs are derived from marrow stem cells that can express antigens corresponding to more than one cell line (12). Classical models of hematopoiesis are based on early separation of a myeloid/macrophage precursor from a common B and T lymphoid precursor (CLP), but alternative models based on intermediate T/myeloid (T/My) and B/My precursors have been proposed (13, 14). In an attempt to identify AL, which might correspond to expansions of intermediate T/My or B/My precursors, Dupret et al suggested that the evidence of early heavy chain immunoglobulin (IgH) DJ and T-cell receptor (TCR) δ/γ rearrangements as early indicators of B and T lymphoid orientation, respectively and TCR rearranged AML may be closely related to T-ALL (15). They also suggested that MLL translocation (t-MLL) and its partners play an important role not only for their co-expression of both myeloid and lymphoid phenotypes, but also for determination of the maturation arrest in AL at the early stages of myeloid/lymphoid specification. [In the present case, this could be formally proven.]

In addition, the malignant transformation or lineage switch of a myeloid or lymphoid leukemic progenitor with the potential to differentiate into another lineage is also thought to be one cause and differential diagnosis of BALs, such as chronic myeloid leukemia (CML) at blast transformation (16) or relapse of the original clone and emergence of a second new clone (17), while no evidence was found in our patient.

According to these considerations, we thought that the origin of his leukemic cells were intermediate T/My precursors differentiating to the myeloid lineage. Therefore, we treated this patient with the conventional induction chemotherapy regimen designed for AML, and he responded well, resulting in a 1st-CR. Because of the poor prognosis of BAL (18), we are planning an intensive consolidation therapy and he will undergo BMT during this first remission.

In summary, BAL is a rare type of AL that is difficult to diagnose and that carries a poor prognosis. Therefore, it is important to elucidate the nature or origin of the neoplastic cells using multi-parameter analysis including morphological, molecular, cytochemical, or immunological assays and perform risk stratification, in order to make appropriate decisions regarding chemotherapeutic treatments to achieve remission, with treatment tailored to the prognostic factors of individual patients. Since BAL is a rare condition, a large number of investigations and co-operation among different institutions will be necessary to carry out these types of studies.
References