Case Study

Diagnostic challenge: Acute leukemia with biphenotypic blasts and *BCR-ABL1* translocation

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Abstract: Chronic myelogenous leukemia (CML) and mixed phenotype acute leukemia (MPAL) are two distinct entities according to the current WHO classification. However, the distinction can be difficult when adequate clinical history is not available, because both entities could have *BCR-ABL1* translocation and B/myeloid biphenotypic blasts. Here we report a 64-year-old man with no previous history of myeloproliferative disorders who presented with marked leukocytosis and 30% circulating blasts. The bone marrow had 39% of blasts and 32% maturing myeloid cells. The blast population showed both B and myeloid differentiation. Cytogenetics revealed the *BCR-ABL1* translocation as the sole abnormality. Based on one small-sized case series, many features of our case favored the diagnosis of CML in blast phase with biphenotypic blasts, including: 1) left-shifted granulocytic maturation in peripheral blood; 2) a significant portion of maturing myeloid cells in bone marrow; 3) the diagnostic marrow with higher percentage of *BCR-ABL1* harboring cells than that of the blasts; 4) *BCR-ABL1* translocation as the sole cytogenetic abnormality; 5) post-induction marrow showed hypercellularity.

Keywords: Acute leukemia, Biphenotypic blasts, *BCR-ABL1*

Introduction

Chronic myelogenous leukemia (CML) is the prototype of myeloproliferative neoplasm, characterized by typical clinical course and a balanced genetic translocation, t(9;22)(q34;q11.2). The t(9;22) fuses the Breakpoint Cluster Region (BCR) gene on chromosome 22q11.2 with the ABL1 proto-oncogene at 9q34, which is also known as the Philadelphia (Ph) chromosome [1]. Majority of CML cases present in chronic phase and the diagnosis is usually straightforward. However, a small portion of CML cases present initially with blast phase (BP), for which the diagnosis can be challenging. Two factors contribute to the diagnostic challenge. First, the blasts of CML could have myeloid, lymphoid or biphenotypic differentiation [2]. Second, Ph can be observed in CML, B-lymphoblastic leukemia (B-LBL) and mixed phenotype acute leukemia (MPAL) or "bipenotypic acute leukemia". Therefore, differential diagnosis for *de novo* Ph+ acute leukemia includes CML in BP, Ph+ B-LBL, and Ph+ MPAL.

Here we report a case of acute leukemia with biphenotypic blasts and *BCR-ABL1* translocation. The patient was a 64-year-old man with no history of myeloproliferative disorder who presented with 30% circulating blasts. The blasts had both myeloid

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and B-cell differentiation. *BCR-ABL1* translocation was the only genetic abnormality. The discussion focuses on the diagnostic dilemma and the distinction between CML in BP versus MPAL.

Case Report

The patient was a 64-year-old man with past medical history of prostate cancer status post radiation treatment, Diabetes Mellitus, and hypertension. He presented to an outside hospital in early 2014 with bilateral foot swelling and was found to have bilateral deep vein thrombi and pulmonary embolism. Peripheral blood smear showed marked leukocytosis with circulating blasts. He was transferred to our institution for further management. The patient's complete blood count was as follows: white blood cells (WBC) of 86.2 $x10^6$/L, red blood cells (RBC) 3.22 x 10^9/L, Hemoglobin 10 g/dL, MCV 97.5 fL, Platelets 210 x 10^6/L.

The peripheral blood smear showed marked leukocytosis with granulocytic left-shifted maturation. There were approximately 30% circulating blasts that showed dimorphic morphology, including smaller-sized blasts with higher nuclear-to-cytoplasmic ratio (Figure 1A, left panel) and a second population of intermediate-sized blasts with fine chromatin, prominent nucleoli, moderate amount of basophilic cytoplasm (Figure 1A, right panel).

The bone marrow biopsy showed marked hypercellularity (>90%) with 39% blasts. The blasts in bone marrow had similar dimorphic morphology as those in peripheral blood (Figure 1B). There were plenty maturing myeloid precursors in the marrow (Figure 1B, 1C). Immunohistochemistry performed on the marrow core biopsy showed that the blasts were positive for CD34 (Figure 1D), CD19 (Figure 1E), CD79a, and TDT. Flow cytometric immunophenotyping showed that 27% of the total cells were blasts with an immunophenotype of CD10 (+), CD13 (dim+), CD19 (+), cytoplasmic CD22 (dim+), CD33 (partial+), CD34 (+), CD45 (dim+), cytoplasmic CD79a (dim+), HLA-DR (+), MPO (dim+), TDT (+), CD3 (-), CD4 (-), CD5 (-), CD7 (-), CD14 (-), CD15 (-), CD56 (-), CD64 (-), CD117 (-).

The karyotype analysis identified t(9;22)(q34;q11.2) as the only abnormalities in all 20 cells (100%) examined. FISH confirmed the presence of t(9;22)(q34;q11.2) by the ASS1-BCR1-ABL probes. The *BCR-ABL1* transcript was the b3a2 or p210 fusion by RT-PCR.

In summary, the blast population showed both myeloid (MPO+) and B lineage (CD19+, cytoplasmic CD79a+, cytoplasmic CD22+, CD10+) differentiation. In the presence of *BCR-ABL1* translocation, the differential diagnosis includes CML in BP with biphenotypic blasts versus MPAL with *BCR-ABL1* translocation under the current WHO classification.

The patient received Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, Adriamycin, dexamethasone) induction with dasatinib plus intrathecal methotrexate prophylaxis. Dasatinib was later switched to imatinib due to intolerance. Day 21 post induction marrow was hypercellular (60-70%) with less than 5% blasts. FISH detected *BCR-ABL1* translocation in 1% of total cells. Day 60 post induction marrow showed cytogenetic remission. Afterwards, the patient reported intolerance and incompliance with the tyrosine kinase inhibitor (TKI) therapy. The 4-month and 6-month post induction marrows were morphologically negative, but showed 4-5% cells harboring *BCR-ABL1* translocation by FISH. Given the poor prognosis and prior complications of chemotherapy, the patient was on maintenance therapy with 200 mg imatinib daily. Later, the patient was transferred to an outside hospital and lost of contact since then.

Discussion

MPAL is a rare subtype of acute leukemia that shows differentiations of two or more hematopoietic lineages and Philadelphia chromosome is the most common recurrent genetic abnormalities in MPAL [3]. According to WHO classification, a history of

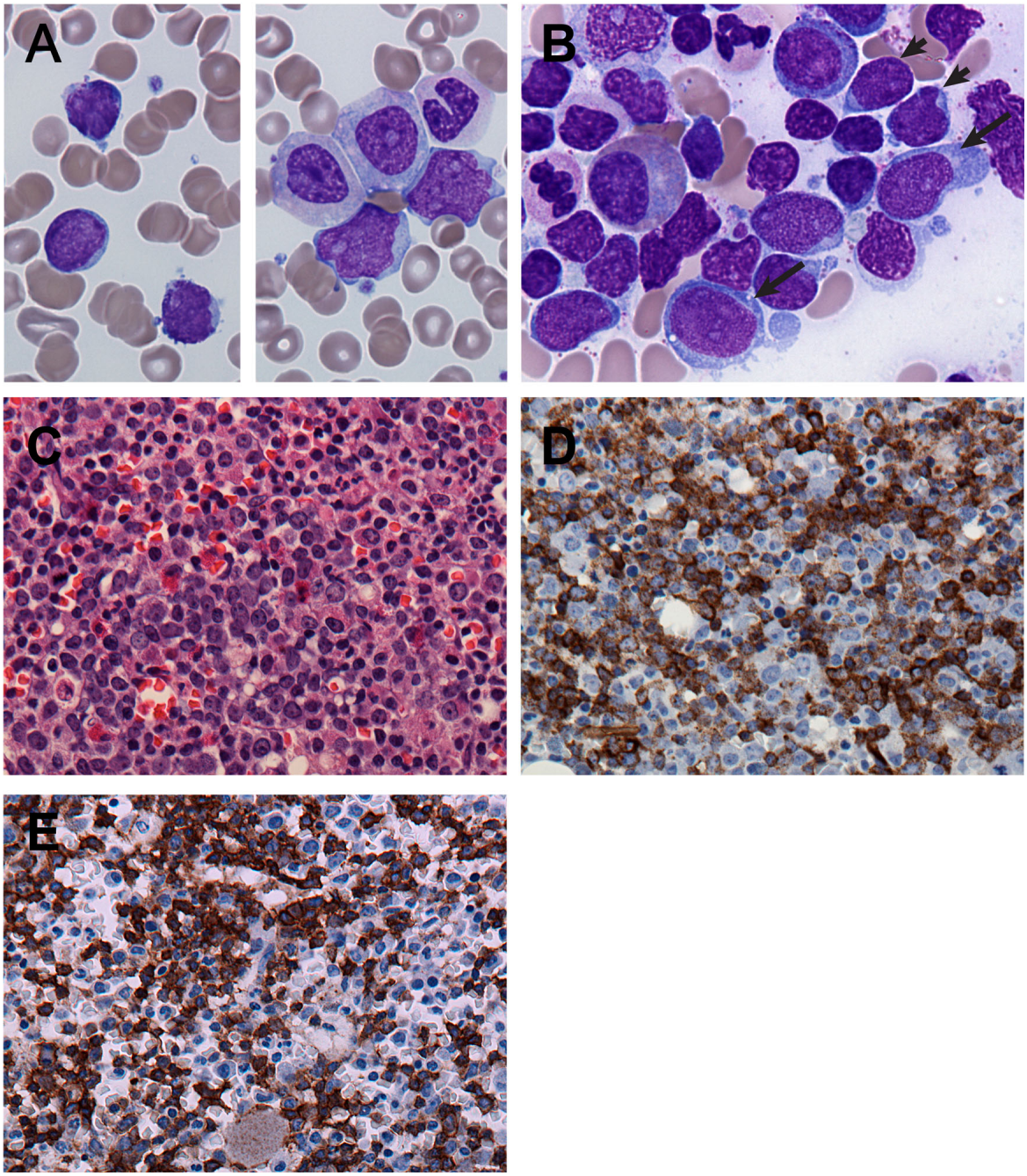

Figure 1: Peripheral blood and bone marrow of the acute leukemia with B/Myeloid biphenotypic blasts. (A) Circulating blasts in the peripheral blood show dimorphic morphology: Left panel shows smaller blasts with high nuclear-to-cytoplasmic ratio and scant cytoplasm. Right panel shows medium-sized blasts with moderate amount basophilic cytoplasm and prominent nucleoli (Wright-Giemsa). (B) Bone marrow aspirate contains the same dimorphic populations of blasts. Short arrow: smaller blasts; long arrow: medium-sized blasts (Wright-Giemsa). (C) Bone marrow biopsy shows increased number of blasts with plenty of maturing myeloid precursors (H&E). The blasts are positive for CD34 (D) and CD19 (E) (A, B: original magnification 1000X; C, D, E: original magnification 400X).

known CML excludes the diagnosis of MPAL. However, when adequate clinical history is not available, there is no consensus or guidelines for distinguishing *de novo* MPAL with *BCR-ABL1* from CML in BP with biphenotypic blasts. Our case is a great example illustrating such a situation.

There are certainly some empirical features helping the distinct. When there is significant myeloid maturation in the marrow, WHO classification suggests using cautions in diagnosing MPAL because MPAL usually shows predominantly blast population with no significant myeloid maturation. Another potentially helpful feature is that MPAL cases typically have additional and complex cytogenetic abnormalities. In contrast, 80% of CML in BP have additional, but often characteristic, abnormalities, including extra Ph, +8, +19, or i(17q) [4]. In addition, some clinicopathologic features are believed to help the differentiation, as discussed in one small-scaled case series with 4 cases of AML with biphenotypic blasts [5]. In particular, the authors believe the following features favor CML BP with biphenotypic blasts: 1) splenomegaly, 2) peripheral leukocytosis with left-shifted maturation at various stages, 3) absolute basophilia, 4) significant proportion of maturing myeloid cells in bone marrow, 5) *BCR-ABL1* as the sole cytogenetic abnormality, 6) the diagnostic marrow with higher percentage of *BCR-ABL1* translocation than that of the blasts, 7) hypercellularity of post induction marrow [5]. Our patient had 5 (No. 2, 4, 5, 6, 7) out of 7 aforementioned features and therefore, CML in BP with biphenotypic blasts is our favored diagnosis.

Of a side note, it is known that the most common type of *BCR-ABL1* transcripts in CML is b3a2 (p210) [6, 7]. In contrast, there is no consensus what are the most common *BCR-ABL1* transcripts in Ph+ MPAL. Therefore, transcript subtypes of *BCR-ABL1* fusion are not useful in the distinction.

In light of the upcoming new WHO Classification, it is worth to mention another related entity of AML: *de novo* Ph+ AML or AML with *BCR-ABL1*. AML with *BCR-ABL1* will be added as a provisional entity in the upcoming WHO Classification 2016 [8] to emphasize the observed efficacy of TKI therapy for these rare AML cases. Studies have shown that *de novo* Ph+ AML have unique molecular signatures separating from other Ph+ entities [9, 10]. It is suggested that loss of certain antigen receptor genes, *IKZF1*, and/or *CDKN2A* would be helpful to diagnose *de novo* Ph+ AML [9]. Since the exact diagnostic criteria for AML with *BCR-ABL1* are not available yet, it is unclear whether our case would fit better under this provisional category and it is interesting to know whether it include cases with biphenotypic blasts.

Our case highlights the difficulty in diagnosing acute leukemia with biphenotypic blasts and *BCR-ABL1* under the current WHO classification of 2008. There are certain suggested morphologic, immunohistochemical, flow cytometric, and cytogenetic features helpful in making such differentiation. The new WHO provisional entity of AML with *BCR-ABL1* is a new factor in stratification Ph+ AML cases and it takes time and efforts to understand its impact on our knowledge of *BCR-ABL1* in AML biology. Newly identified molecular markers may offer a more objective way to distinguish among Ph+ acute leukemias under the upcoming new classification.

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