Case Study

Central nervous system relapsed B-Lymphoblastic Leukemia with t(9;22)(q34;q11) *BCR/ABL1* mimicking acute myeloid leukemia

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Abstract: The presence of the Philadelphia (Ph) chromosome is one of the worst prognostic factors in B-cell acute lymphoblastic leukemia (B-ALL). Cerebrospinal fluid (CSF) involvement is a high-risk factor and serious complication in patients with B-ALL. Here we report a 21-year-old female diagnosed with Ph positive B-ALL and subsequently developed CSF relapse and rapid demise. Her CSF contained numerous blasts resembling myeloblasts. The relevant clinicopathologic distinctive scenarios and differential diagnoses are discussed.

Keywords: B-lymphoblastic leukemia (B-ALL), Blasts, Cerebrospinal Fluid, Philadelphia chromosome

Introduction

The *BCR-ABL1* fusion gene located on the Philadelphia (Ph) chromosome is found essentially in all myeloid lineages, erythrocytes, megakaryocytes and lymphoid cells. It is one of the most common cytogenetic abnormalities in chronic myeloid leukemia (CML) and acute lymphocytic leukemia (ALL). Leukemic infiltration of the cerebral spinal fluid (CSF) is characterized as five or more leukocytes/mm^3 and the presence of blasts in CSF. The cumulative incidence of central nervous system (CNS) relapse varies considerably in B-ALL patients. However, no reliable morphological CSF involvement is evident at diagnosis in about 60% of patients who eventually develop CNS relapse. Multiple risk factors, such as genetic alterations t(9;22) and mixed lineage leukemia (*MLL*) rearrangements, have been associated with a higher incidence of relapse in CSF. When acute myeloid leukemia (AML)-like blasts are present in CSF, several entities enter the differential diagnoses. Here we report a case of AML-like blasts involving CSF in a B-ALL patient with Ph+ t(9;22)(q34;q11) *BCR/ABL1*.

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Case Report

This patient is a 21-year-old female who initially presented with epistaxis, severe back pain and fatigue. She was found to be neutropenic with increased blasts and subsequently diagnosed with B-ALL with t(9;22)(q34;q11) *BCR/ABL1* (described in Pathologic and Genetic Findings). She received 8 cycles of hyper-CVAD with rituxan and dasatinib and achieved remission. However, three months later, the patient complained of frontal headache

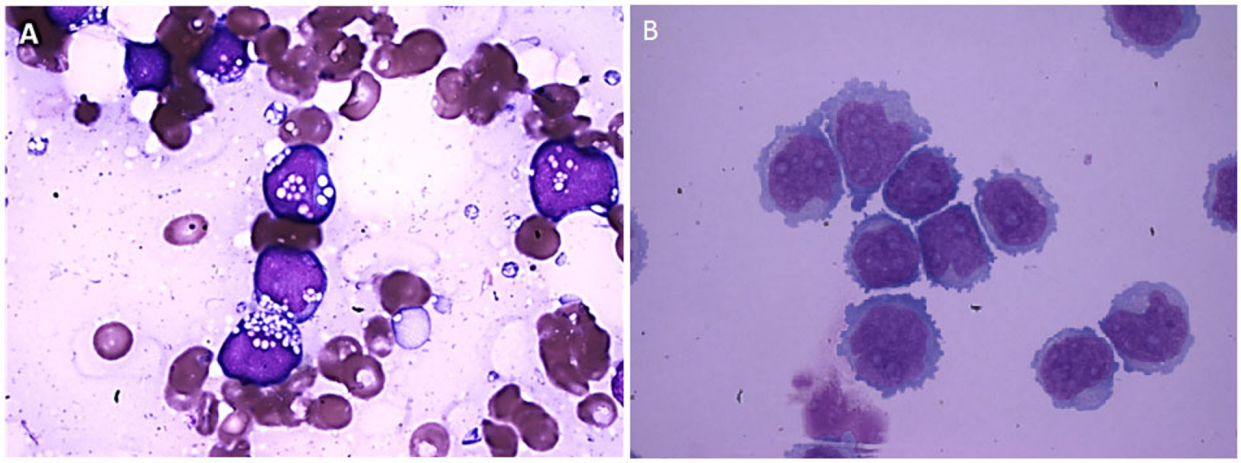

Figure 1: A large population (~90%) of blasts was identified in the bone marrow aspirate smear (A) and cerebral spinal fluid (B) from this patient.

with auras. Lumbar puncture performed demonstrated an increased intracranial pressure and CSF analysis revealed increased white blood cell count of 361. She received intrathecal Methotrexate along with augmented Hyper-CVAD+Dasatinib. After two months of treatment, her CSF showed "rare immature mononuclear cells with undetermined significance". Since then, the patient had multiple relapses while undergoing various therapies, including triple intrathecal therapy (methotrexate, cytarabine, corticosteroid), craniospinal radiation and R-MOpAD based clinical trial. Although radiologic work-up was unremarkable, relapse was confirmed by a large population (98%) of blasts in CSF (described in Pathologic and Genetic Findings). As this portended a poor prognosis, bone marrow transplant was deemed as a futile treatment option. The patient succumbed to her disease one month later.

The bone marrow biopsy showed fibrotic hypocellular marrow with aplastic hematopoiesis and a large population of blasts (~90%). The blasts were medium-sized with prominent invaginated nuclei, fine nuclear chromatin, conspicuous nucleoli and moderate amount of basophilic agranular vacuolated cytoplasm [Figure 1A]. Flow cytometry demonstrated dim CD45, HLA-DR, CD34, dim CD38, CD19, CD10, heterogenous CD20, TDT, CD22 and CD79a without T-cell or myeloid antigens consistent with B-ALL. Similar findings were seen in the subsequent CSF relapse [Figure 1B]. Cytogenetic analysis of bone marrow cells showed a very complex abnormal female karyotype: 47, XX, del(3)(p21), del(6)(q13q21), t(8;9)(q13;p13), der(9)del(9)(p13)t(9;22)(q34.1;q11.2),der(22)t(9;22),+mar [4]46,XX[16]. Four of the 20 cells analyzed showed multiple chromosome alterations, including t(9;22)(q34.1;q11.2) with the derivative 9 containing a deletion of its short arm. FISH analysis confirmed t(9;22) or *BCR/ABL1* fusion and additional copies of chromosome 4.

Discussion

ALL is one of the most common malignancies in patients under 20 years of age [1]. The majority (85-90%) are B-cell lineage [2]. While 5-year event-free and overall survival rates are high in children and young adults [3], adults with B-ALL have a significantly worse prognosis than the pediatric counterparts, which may be explained by the more complicated genetic alterations [3]. The Ph chromosome is formed as a result of t(9;22)(q34;q11) and is one

of the most common cytogenetic abnormalities in CML and ALL. Patients with Ph+ ALL belong to a readily diagnosable, distinct subgroup comprising 20 to 30% of adults and 2 to 3% of children with ALL. In contrast to CML and AML cases, complex karyotypes and hyperploids are frequently seen in B-ALL with t(9;22)(q34;q11). Additional cytogenetic anomalies are found in 50 to 80% of cases of B-ALL, such as +der(22), -7, del(7q), most often +8, but not i(17q) [4].

Occurring in 90-95% of CML and 20-30% of the adult ALL, t(9;22) *BCR-ABL1* fusion gene results from the fusion of *BCR* at 22q11.2 and cytoplasmic tyrosine kinase of *ABL1* at 9q34. In most pediatric cases of t(9;22) ALL, a p190 kd *BCR-ABL1* fusion protein is produced. In adults, about one half of the ALL with t(9;22) produce p210 kd that is present in CML, with the remainder producing p190 [5]. Some reports suggest that the cell of origin for t(9;22) is more immature than that of other B-ALL cases [5]. ALL patients with t(9;22) have the worst prognosis and may be associated with other genetic abnormalities, which lead to unusual morphologic features/immunophenotypes and aggressive clinical behavior [5].

The Ph chromosome is present in approximately 20 to 30% of adult ALLs. While Ph+ ALL will respond to combination chemotherapy, complete remission is significantly less likely than Ph- ALL. The remission duration is short (median event-free survival is approximately 8 months) and the prognosis is poor. Despite a relative paucity of randomized clinical trials, the poor prognosis of this relatively uncommon acute leukemia has led to the rapid adaptation of treatment strategies such as tyrosine kinase inhibitors (TKI) and unrelated donor hematopoietic stem cell transplant. Our patient had multiple CNS relapses and aggressive clinical course. A substantial portion of the CSF blasts have distinctive nuclei with prominent clefts or invaginations, fine nuclear chromatin, conspicuous nucleoli and moderate amount of basophilic agranular vacuolated cytoplasm, features that are reminiscent of myeloblasts. However, immunophenotypic results from flow cytometric analysis support a B-ALL.

The 2008 World Health Organization scheme defined distinct categories of B-ALL by immunophenotyping with subtypes substantially amplified by cytogenetics and molecular diagnostic tools. Other variants of B-ALL may include cytoplasmic granules, aplastic presentation, eosinophilia, relapse of lymphoblastic leukemia and secondary ALL. In the current case of CNS relapse of B-ALL, the morphological differential diagnosis includes the following:

1. "B lymphoblast crisis" of CML with CNS relapse. Although rare, this is an important clinical scenario to consider, as the initial CML may be indolent and undiagnosed. When blast crisis occurs, myeloblasts are more common, consisting 70% of the cases. However, 20% can present as B lymphoblasts, with occasional cases of T lymphoblastic, monoblastic, megakaryoblastic, erythroblastic and multilineage blastic crises [6, 7], all carrying worse prognosis. Pattnaik *et al*. reported a case of t(9;22) CML presented as blast crisis mimicking ALL in a child. The morphologic presence of basophils and basophilic myelocytes can be seen in peripheral blood and lymph node aspirate smears; however, pre-B cell lineage by immunomarkers in flow cytometry played an important role in diagnosing the case as CML with lymphoblastic crisis [8]. At the molecular level, the most common mutations detectable at the loci of cyclin-dependent kinase inhibitor 2A/2B (*CDKN2A/B*) (50% of cases) and Ikaros transcription factor (*IKZF1*) (55% of cases) can be seen in lymphoid transformation of CML [9–11]. Homozygous deletion of p16 tumor suppressor gene is also associated with lymphoid transformation of CML [12].

2. Biphenotypic acute leukemia. In cases with dimorphic blast population, the possibility of a mixed phenotype acute leukemia with t(9;22) (q34; q11.2) should be considered. This is a very rare form of leukemia (<1%) and patients usually presented with high white blood cell count, similar to those with Ph+ ALL. Our patient did not have a mixed phenotype on flow cytometric analysis.

3. "Cup-like" leukemic cells in AML. To some extent, the leukemia cells in our case resemble cup-like leukemic cells in AML. The cup-like nuclear phenotype was defined by the presence of nuclear invaginations in ≥25% of the nuclear diameter. Myeloperoxidase activity can be observed in a distinctive granular pattern within the areas of nuclear invagination and electron microscopy revealed an accumulation of mitochondria in the nuclear indentation. This morphology is associated with high blast counts in the peripheral blood and bone marrow, especially in AML M1 (former FAB classification). Gene mutation of both *NPM1* and *FLT3-ITD* or TKD strongly correlated with the cup-like nuclear morphology. Although it is well recognized that blasts with cup-like nuclei are associated with AML, including AML with t(9;22)(q34;q11.2), Hu *et al*. reported that they can be encountered in B-ALL [13]. The morphology of clefted-nuclei looking monoblasts and promonocytes overlaps with that of cup-like nucleated cells. Therefore, AMLs (M4 and M5) easily enter the differential diagnosis as these entities often associated with CNS involvement.

4. Burkitt leukemia, or L3-ALL (former FAB classification). Typical L3 blasts are medium to large in size and homogeneous. The nuclei are regular and round to oval in shape. One or more prominent nucleoli are present. The amount of the cytoplasm is moderate and contains prominent vacuoles. Cytochemistry of L3 blasts is always negative for MPO and NSE, while intensely positive for cytoplasmic methyl green pyronine; cytoplasmic vacuoles stain strongly with Oil red O [14]. All L3 leukemias are surface immunoglobulin (sIg) positive and are of B cell lineage. A leukemic phase can be seen in patients with bulky disease of Burkitt lymphoma, but rare cases can be present purely as acute leukemia with peripheral blood and bone marrow involvement [5]. CNS involvement is common in L3 ALL, thus influencing both treatment and prognosis. Recognizing this entity is important as patients with L3 morphology showed improved outcomes when treated with specific algorithms [15, 16]. Some studies found that L3 leukemia can be cured with aggressive, rapidly cycling lymphoma-like chemotherapy regimens [15, 17, 18]. L3 ALL is associated with a variety of translocations including the *c-MYC* proto-oncogene to the immunoglobulin gene locus t(2;8), t(8;12), and t(8;22). In fact, translocations involving chromosome 8 (*MYC* gene) are present in 100% of B-ALL with L3/Burkitt morphology and clonal mature sIg [15]. In our patient, cytogenetic studies showed a very complex karyotype with multiple chromosome changes, most critical of which is the t(9;22)(q34.1;q11.2). Another important translocation is t(8;9) that contains alterations in chromosome 8. We speculate that such combination of unusual genetic abnormalities may have led to the unique morphology of "AML-like" blasts in our patient. In addition, other genetic abnormalities such as deletion of 9p are also an adverse risk factor for B-ALL [19]. Additional copies of chromosome 4 are also present with unclear clinical significance. All of these genetic abnormalities may have contributed to the aggressive clinical behavior and rapid demise in this patient. The co-existence of t(9;22)(q34;q11) and t(8;9)(q13;p13) is rare. Some studies have suggested that the occurrence of a second translocation involving chromosome 8 in addition to *BCR-ABL1* is strongly associated with advanced stage of disease, as an additional growth advantage of leukemic cells is conferred [20]. Our patient may have experienced blast crisis/relapse due to harboring both clones when both translocations are present.

Our patient has received Ponatinib, a multi-targeted TKI. This type of drug was design for the treatment of CML and Ph+ ALL. To what extent the treatment with TKI contributed to the acquisition of t(8;9) remains elusive. It is unclear if t(8;9) arisen de novo or secondary to TKI treatment in this patient. Therefore, when TKIs are considered as a treatment option for CML patients harboring both t(9;22) and a second translocation involving chromosome 8, when the responses are suboptimal, they should be used only briefly as patient awaiting allogeneic HSCT [20].

There is growing consensus that identifying factors that increase risk of leukemic CNS involvement can lead to early modification of protocols aiming at treatment and CNS prophylaxis. It is well established that leukemic patients can benefit from concurrent cytology and flow cytometry studies, as patients with any positive result from either study may receive early intervention, even when they are clinically asymptomatic.

In summary, blasts in CNS relapsed B-ALL with t(9;22)(q34;q11) *BCR/ABL1* can mimic myeloblasts in AML. The aforementioned differential diagnoses should be considered when encountering challenging scenarios similar to our case. The combination of morphologic, immunophenotypic and cytogenetic/molecular studies is the best approach to render an accurate diagnosis.

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