Diagnostic Challenges of *De Novo* Acute Leukemia with Positive BCR-ABL1 Fusion: Case Report and Review of Literature

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Abstract:
A 32-year-old female was admitted because of an acute course of gum bleeding and fatigability. On complete blood cell counts, bicytopenia was evident. Morphologic evaluation of the peripheral blood smear and bone marrow materials revealed remarkable involvement by two separate blastic populations (myeloid/B-cell lymphoid). Karyotyping, fluorescence *in situ* hybridization, and molecular analysis showed positive Philadelphia chromosome and BCR-ABL1 (p210) fusion. chronic myeloid leukemia in blast phase (CML-BP) versus mixed phenotype acute leukemia was critically debated as to which the final diagnosis should be assigned.

Keywords:
Acute leukemia, BCR-ABL1 + acute leukemia, blast crisis, chronic myeloid leukemia, mixed phenotype acute leukemia

Introduction
Among acute leukemic cases, *de novo* BCR-ABL1 + mixed phenotype acute leukemia (MPAL) is a rare disease accounting for <1%. However, it is considered the most common recurrent genetic abnormality identified in such a group of leukemias.[1,2]

In the context of a negative record of preceding leukocytosis or splenomegaly, *de novo* BCR-ABL1 + acute leukemia (AL) may be overlooked as a differential diagnosis to chronic myelogenous leukemia in a primary blast crisis (CML-BC). In such context, patients with CML-BC should be distinguished from other patterns of AL because of its distinct pathophysiology and potentially different therapeutic approach.[3,4]

Case Report
A 32-year-old female had acutely presented to the emergency department at the end of 2017. The anonymous reporting of this case was approved by the local bioethical committee of the medical city. Her primary complaint was gum bleeding and easy fatigability over a week. The past history was unremarkable. The physical examination revealed only mild splenomegaly.

The initial complete blood cell results showed hyperleukocytosis (189.7 × 10^9/L) along with significant blastemia, severe anemia (6.2 g/dL), and thrombocytopenia (42 × 10^9/L). AL, as a diagnosis, was entertained and bone marrow aspirate and trephine biopsy were subsequently obtained for further assessment.

The peripheral blood film showed marked leukocytosis with around 70% circulating blasts and left shift. Blasts...
formed morphologically of two distinctive populations. The first population constituted 39% and resembled lymphoblasts (small to medium in size, very high nuclear/cytoplasmic (N/C) ratio, open chromatin, inconspicuous nucleoli, scanty cytoplasm, and absent Auer rods). The second population represented 31% and assumed the morphology of monocytic precursors; 24% appeared monoblastic in nature (medium to large in size, moderate N/C ratio, open chromatin, prominent nucleoli and variably abundant basophilic cytoplasm that contained, sometimes, fine azurophilic granules and a few vacuoles but no Auer rods were seen), whereas the remaining 7% seemed more to be of promonocytic stage that exhibited more irregular and delicately folded nucleoli, with fine chromatin, small indistinct nucleoli, and not infrequent fine cytoplasmic granules and vacuoles. With regard to granulocytic series, about 15% promyelocytes of unremarkable morphology were appreciated. Myelocytes, metamyelocytes, and bands/polymorphonuclear neutrophils accounted for 3%, 4%, and 5%, respectively. No clear bulge at either myelocytic or mature neutrophilic stages was noted. Eosinophils and basophils accounted for 2% and 1%, respectively. Morphology of red blood cells (RBCs) reveals marked anisocytosis and moderate poikilocytosis with infrequent schistocytes and rare nucleated RBCs. Severe thrombocytopenia was evident [Figure 1].

**Bone marrow aspirate**

This sample, which was obtained from the left posterior iliac crest, contained few tiny, highly packed particles with very cellular trails and occasional unremarkable megakaryocytes. 500-cell differential counting was carried out revealing a major blastic pool of 79.4% of all nucleated cells, namely 30% lymphoblasts, 36.6% monoblasts, and 12.8% promonocytes. Their morphology was compatible with what described in the peripheral blood film. Erythropoiesis was markedly suppressed (1% of all nucleated cells). Granulopoiesis accounted for 16.6% appeared mildly shifted and morphologically unremarkable. Both eosinophilic and basophilic series were not increased. Lymphocytes constituted 3%.

**Flow cytometric analysis**

A peripheral blood specimen was processed for 4-color immunophenotyping using FACSCanto II and analyzed with CD45– side scatter, using FACSDiva 6.3 version software (Becton, Dickinson and Company, BD Biosciences, San Jose, CA, USA). The acquisition rate of events was 10,000 events/tube. It showed two distinctive populations. The first group accounted for 31% of all events, fell in the blast gate (dim CD45/low SSC) and expressed TdT, CD34, HLA-DR, CD79a, CD19, CD22, CD10, and partially CD13, and lacked other lineage-specific markers. The second population, which represented about 42% of all events, were characterized by bright CD45 and slightly increased SSC and were positive for CD64 (bright), MPO, TdT (partial), CD13, HLA-DR (partial), CD33, CD14 (partial), CD36 (partial), CD15, CD11b, and CD11c [Figure 2].

The submitted bone marrow trephine biopsy was suboptimal, composed of about 0.6 cm crushed/fragmented bone marrow core biopsy along with a large attached blood clot, showing very hypercellular marrow (almost 100% cellularity) with extensive infiltration by immature cells (blasts) of variable size at the expense of normal hematopoiesis.

Cytogenetic analysis of the bone marrow cells revealed 46,XX, t(9;22)(q34;q11.2)[20] in all metaphases analyzed. Multiplex-nested reverse transcription-polymerase chain reaction assay for BCR-ABL1 (p. 210 kd) was positive, but appeared negative for RUNXI-RUNX1T1, CBFB-MYH11, PML-RARA, KMT2A-MLLT3, DEK/NUP214, GATA2-MECOM, RBM15-MKL1, ETV6-RUNX1, IGH-IL3, TCF3-PBX1, FLT3-ITD, KMT2A-AF4, and KMT2A-MLLT1. With regard to fluorescence in situ hybridization (FISH) analysis, dual-color, dual-fusion probes for BCR-ABL1 exhibited one fusion, one red, and one green signals (1F1R1G), indicative of deletion of the derivative chromosome 9 involving sequences 5’ of the ABL1 breakpoint and 3’ of the BCR breakpoint. This abnormality was ubiquitous in all examined cells including mature neutrophils [Figure 3]. Other probes for PML-RARA and KMT2A rearrangement were negative. Mutational analysis for CEPBA, FLT3-ITD, FLT3-TKD, and NPM turned out to be negative, as well.

In brief, the immunomorphological findings, cyogenetics, FISH, and molecular analyses of the submitted specimens...
are in favor of chronic myeloid leukemia in primary blast crisis (CML-BC) with mixed immunophenotypic profile (myeloid/B-cell lymphoid), “46,XX,t(9;22)(q34;q11.2) [20]” karyotype and +ve BCR-ABL1 (p. 210 kd) transcript.

**Discussion**

Typically, untreated CML runs a triphasic course, progressing from the chronic phase to accelerated stage and eventually culminating into blast crisis. However, blast crisis of CML can be the up-front manifestation of the disease with a varied rate of 0.9%–6.7%.[5-7] The WHO criteria for the blast phase of CML include either “(1) ≥20% blasts in the blood or bone marrow or (2) extramedullary blastic infiltrations.” Table 1 highlights the antigenic profile necessary for assigning blasts to...
either myeloid, T- or B-cell lineages as per the blue monograph of the classification of hematolymphoid neoplasms.[8]

In our case, the blasts fulfill the WHO immunophenotypic criteria for assigning leukemic blasts to both myeloid (with monocytic differentiation) and B-cell lineages qualifying it as a MPAL as highlighted in Table 1.[8] Given the positivity for t (9;22)(q34;q11.2) combined with unremarkable past history in our case, the diagnosis of de novo MPAL with such a recurrent genetic abnormality is an essential differential diagnosis, yet still we have to exclude CML in primary blast crisis (CML-BC) first. Especially with the lack of previous history of visceromegaly or leukocytosis, the discrimination between CML-BC and de novo BCR-ABL1+ MPAL is challenging.

Although our case is not purely of myeloid lineage, a diagnostic algorithm proposed by Neuendorff et al. primarily for differentiating de novo BCR-ABL1+ from CML-BC acute myeloid leukemia might be exploited in our scenario. They took into their account particular parameters pertaining to history and morphologic and molecular findings, including “antecedent blood anomaly, signs of myelodysplastic syndrome (MDS), basophilia, BCR-ABL1 transcript product, and number of BCR-ABL1+ metaphases.”[9] In our case, lack of splenomegaly and percentage-wise basophilia hampered such a differentiation between de novo BCR-ABL1+ and CML-BC MPAL. However, the 100%‑positive Ph chromosome in the whole composite of the examined metaphases along with the exclusive detection of p210 BCR-ABL1 transcript gave a convincing CML-BC diagnosis.[8,11] In such a context, it is worth glimpsing at the dissimilar pathophysiology implicated in pathogenesis of such entities at the genetic level. Actually, it is accepted that de novo AL (particularly myeloid) undertake a heterogenous multistep process to manifest overtly in which eventually a synergism between two classes of mutations (i. e., I and II) should happen.[12] In other words, BCR-ABL1 alone could not induce de novo overt AL. On the other hand, that genetic defect is the whole mark and sole driver event in CML, adequate for the disease inception. Therefore, “less than 100% of metaphases expressing the Philadelphia chromosome” is a yardstick for diagnosing de novo BCR-ABL1+ AL.[10,13]

In summary, both cytogenetic and molecular findings of our case backed up the diagnosis of CML-BC rather than BCR-ABL1+ MPAL. Although the overall outlook of BC-CML is poor, the primary goal in treating such cases is to reconstitute chronic phase and proceed to allo-SCT as soon as possible. According to the phenotypic profile of leukemia, treatment of CML in primary blast crisis usually encompasses monotherapy with a tyrosine kinase inhibitor that can also be combined with chemotherapy.[3] In our case, given the absent preceding history of CML and splenomegaly, the diagnosis of “de novo MPAL (myeloid/B-cell lymphoid)” was initially proposed. In fact, MPAL is a rare entity representing < 4% of all AL cases.[2,14] Till now, no consensus guidelines are available to inform the clinical practice in terms of their management due to lack of prospective, controlled trials. The limited available data recommend an “acute lymphoblastic leukemia-like” regimen, followed by allogeneic stem-cell transplant beside a tyrosine kinase inhibitor in patients with t (9;22).[13] As a result, an acute lymphoblastic leukemia-like induction chemotherapy in combination with a dasatinib was initiated. After gathering all other cytogenetic and molecular findings, the diagnosis was revised and the diagnosis of “CML in primary BC with mixed immunophenotypic profile (myeloid/B-cell lymphoid)” was introduced to the treating team who believed that their management plan would not be changed accordingly. After achieving complete remission, the patient was referred to a higher center for allo-SCT from an HLA-matched donor and posttransplant maintenance on dasatinib for neuroprophylaxis as it is known to cross the blood–brain barrier.

Table 1: Requirements for assigning more than one lineage to a single blast population[8]

| Lineage                  | Criteria                                                                 |
|--------------------------|---------------------------------------------------------------------------|
| Myeloid lineage          | MPO (by flow cytometry, immunohistochemistry, or cytochemistry), or monocytic differentiation (<2 of the following: Nonspecific esterase, CD11c, CD14, CD64, and/or lysozyme) |
| T-cell lineage           | Cytoplasmic CD3 (by flow cytometry with antibodies to CD3 epsilon chain. Immunohistochemistry using polyclonal anti-CD3 antibody may detect CD3 zeta chain, which is not T-cell-specific), or surface CD3 (rare in mixed-phenotype acute leukemias) |
| B-cell lineage (multiple antigens required) | Strong CD19 with ≥1 of the following (strongly expressed): CD79a, cytoplasmic CD22, and/or CD10, or, weak CD19 with ≥2 of the following (strongly expressed): CD79a, cytoplasmic CD22, and/or CD10 |

Figure 3: Fluorescence in situ hybridization signal pattern of dual-color, dual-fusion probes used in this case demonstrated one fusion, one red, and one green signals.
barrier with regular monitoring of BCR-ABL transcript levels afterward.[3,16]

**Conclusion**

Before labeling a case as *de novo* BCR-ABL1 + AL (either myeloid or MPAL), an extreme caution should be exercised not to miss primary blast phase of CML by undertaking a meticulous cytological examination and detailed cytogenetics and molecular analyses, even in the context of suggestive history.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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