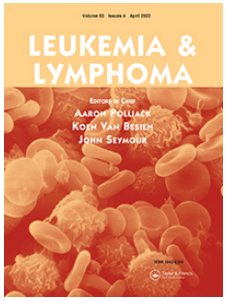

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LETTER TO THE EDITOR

Significance of *RUNX1* mutation in *BCR-ABL1* positive acute myeloid leukemia – a diagnostic dilemma in a young woman with persistent bleeding

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Large-scale genomic analyses of acute myeloid leukemia (AML) have led to extensive molecular characterization of its diagnosis and sub-classification. The 2016 World Health Organization (WHO) classification recognizes a diverse group of AMLs based on characteristic genetic alterations, including chromosomal translocations, insertions/deletions, and single nucleotide variants (SNVs) [1].

De novo BCR-ABL1+ AML and *RUNX1* mutated AML are two provisional entities in the updated 2016 WHO Classification [1]. AML with two specific molecular events, such as *BCR-ABL1* and *RUNX1*, is unusual. *De novo BCR-ABL1*+ AML is a rare, aggressive disease that has many overlapping features with chronic myeloid leukemia in blast phase (CML-BP). While clinical and genetic features may help differentiate *de novo BCR-ABL1*+ AML from CML-BP, there are no defined criteria to distinguish between these two conditions despite significant therapeutic implications. Consequently, it may be challenging to make a diagnosis of *BCR-ABL1*+ AML in the absence of a prior history of hematologic malignancy or leukocytosis. Due to overlapping clinical and genetic features between the two conditions, the identity of *BCR-ABL1*+ AML as a distinct entity is controversial.

Somatic and germline mutations of *RUNX1* are frequent in hematologic malignancies. AML with *RUNX1* mutation accounts for 10% of newly diagnosed patients [2]. *RUNX1* mutations are also found in CML and linked to disease progression and inferior treatment response [3,4]. *RUNX1* germline mutations are associated with familial platelet disorders with a predisposition to hematologic malignancies (FPDMM) [5].

We report the clinical, pathologic, and genetic findings of a young woman who presented with AML with two genetic alterations defined by the WHO as provisional entities and discuss the differential diagnosis, treatment, compare the genomic characteristics of *de novo BCR-ABL1*+ AML and CML-BP and highlight the observation that *RUNX1* mutations are relatively common in both. Further, we discuss recent studies reporting the frequency of *RUNX1* germline variants in AML and emphasize that in many cases, monitoring of *RUNX1* mutation variant allele frequencies (VAFs) may be a prudent way to determine its somatic nature in a subset of patients.

A 27-year-old female, without any significant past medical history, presented with a one-day onset of dyspnea, fatigue and a syncopal episode which brought her to medical attention. Her clinical course was further marked by neutropenic fevers, *C. difficile* diarrhea and a skin infection. She also developed heavy vaginal bleeding that persisted for one month, did not respond to conventional treatment and required blood transfusion. A significant clinical detail was the fact that both her paternal grandmother and great-grandmother had died of acute leukemia of unknown type.

Initial lab work revealed bicytopenia (WBC – 8.3×10^9/L, hemoglobin – 6.9 g/dl, platelets 33×10^9/L) with 45% circulating blasts and LDH levels of 1689 U/L. Prothrombin time and partial thromboplastin time were normal. Platelet function studies were not performed. CT scan showed a spleen infarction. Bone marrow aspirate showed 70% variably sized blasts (Figure 1(A)) with irregular nuclei, fine chromatin, prominent nucleoli, basophilic cytoplasm with occasional vacuoles without any dysplasia. The bone marrow biopsy revealed sheets of myeloperoxidase(subset) and lysozyme(subset) positive blasts that were negative for CD2, CD3, CD1a, PAX5, and TdT. Flow cytometry identified a 51% CD45(dim) population that expressed CD34, CD38, HLA-DR, CD117(subset), CD123, CD1(dim), CD33, CD43, CD56(dim), CD7(dim) and negative MPO, CD11b, CD14, CD15, CD64, T and B cell markers (Figure 1(B)).

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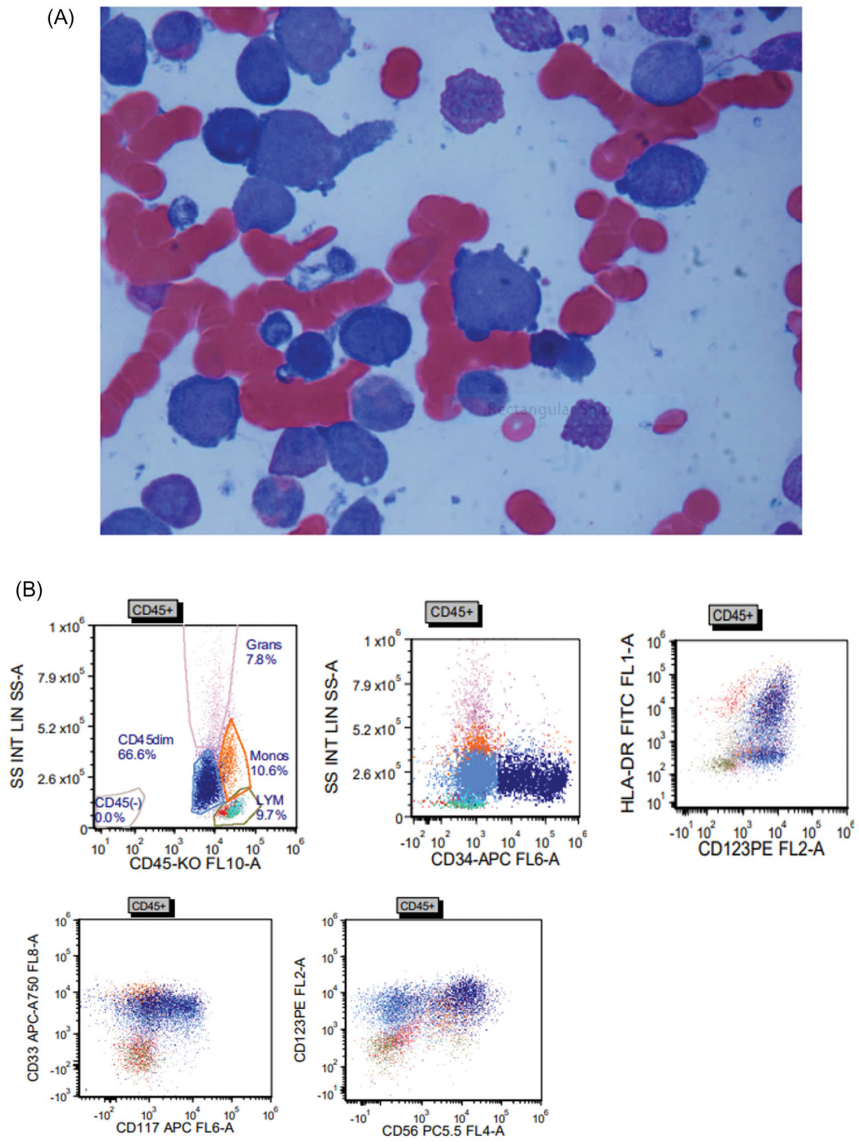

Figure 1. Bone marrow aspirate and flow cytometry findings.

Karyotyping on peripheral blood revealed the following complex karyotype (2A): 56,XX,+6,+8,+9, t(9;22)(q34;q11.2)x2,+10,+12,+13,+19,+21,+22,+der(22)-t(9;22)[11]/57,sl,+19,-der(22)t(9;22)x2,+ider(22)(q10) t(9;22)x2[7]/58,sdl,+21[2].ish der(9)t(9;22)(ABL1+,BCR+)x2,der(22)t(9;22)(BCR+,ABL1+),ider(22)(q10)t(9;22)(BCR++,ABL1++)x2[cp2]/+8(RUNX1T1+),+21(RUNX1+),+21(RUNX1+)[1]

The karyotype revealed three related clones (tetrasomy 19 (7/20 cells) and as tetrasomy 21 (2/20 cells)) suggestive of clonal evolution.

Fluorescence *in situ* hybridization (FISH) performed on the bone marrow aspirate (Figure 2(B)) identified a *BCR-ABL1* fusion (172/200, 86%), trisomy 6, 8, and 10 (62.5%, 72%, and 59.0%, respectively). RT-PCR on peripheral blood revealed a BCR-ABL1 transcript >50% IS (International Scale).

Next-generation sequencing identified a disease-associated *RUNX1* mutation at amino acid 201 in exon 6, resulting in premature termination of the coding sequence in 2421 of 4327 total sequence reads, for a variant allele fraction (VAF) of 56%. The *RUNX1 p.R201** variant is a polymorphism associated with an increased hereditary risk for cancer. A missense variant in *CDH2* (VAF of 57%) was additionally identified as a variant of undetermined significance (Figure 2(C)). A diagnosis of *de novo BCR-ABL1*+ AML with *RUNX1* mutation was made. CML-BP with *RUNX1* mutation or two distinct AMLs with

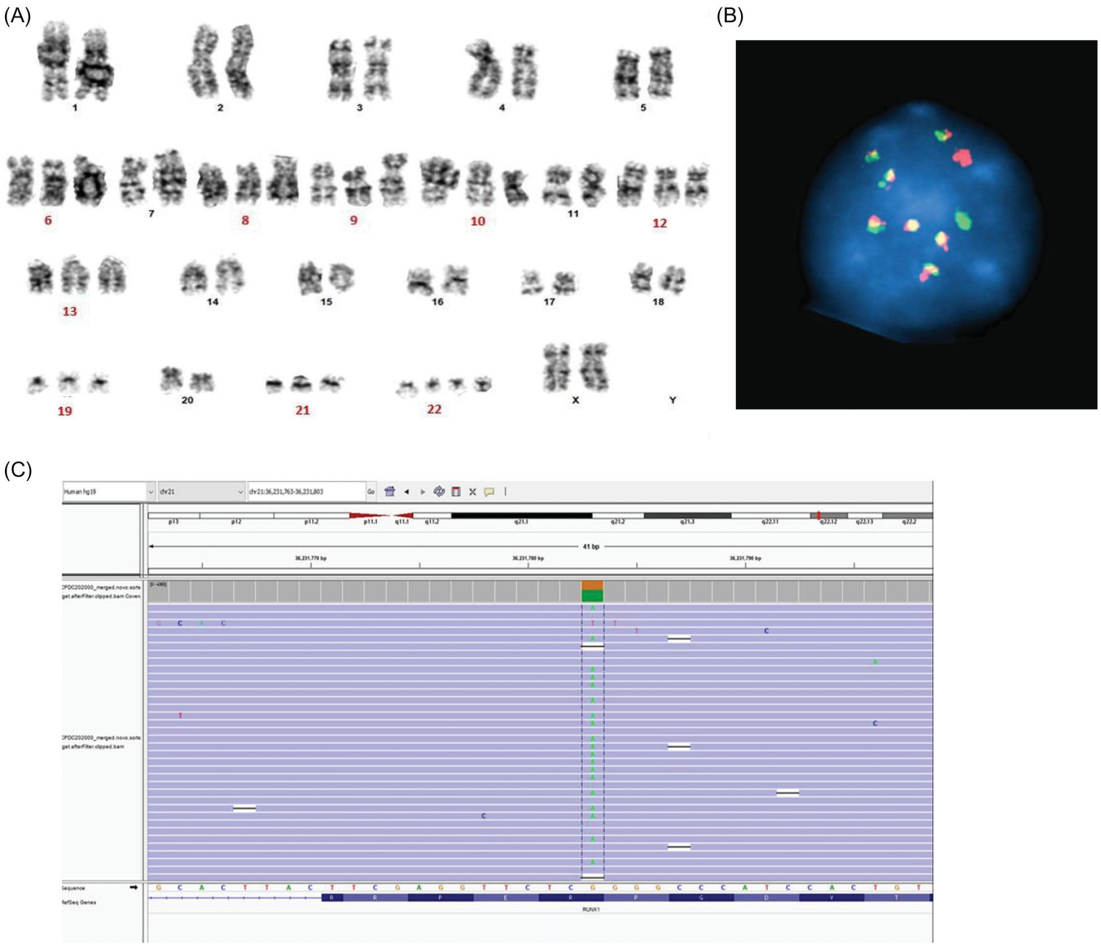

Figure 2. Karyotyping, FISH and gene sequencing results.

two genetic aberrations were alternate diagnostic considerations.

The patient was treated with an induction regimen of '7 + 3' (cytarabine and daunorubicin) with dasatinib. Post induction karyotype, FISH, and gene sequencing studies were normal with no *RUNX1* aberrancy identified, ruling out a germline mutation. Post induction *BCR-ABL1/ABL1* international scale (IS) % was 0.282. The patient was referred for bone marrow transplantation.

De novo BCR-ABL1+ AML is considered a provisional entity in the 2016 WHO Classification as patients may benefit from tyrosine kinase inhibitor (TKI) therapy [6–8]. *RUNX1*-mutated AML is considered a provisional entity to recognize that it may represent a biologically distinct group with a possibly worse prognosis than other AML subtypes [1,9].

Our case demonstrates several challenges regarding its sub-classification. In a young woman without an antecedent history of CML but with a reported family history of hematologic malignancy and bleeding, the salient questions were: is the disease representative of CML-BP or de novo AML with BCR-ABL1, and does the RUNX1 mutation raise the possibility of familial platelet disorder with myeloid malignancy (FPDMM)?

*BCR-ABL*1+ AML is most often seen in AML-NOS, AML with CBF mutation, and AML with myelodysplasia-related changes. It is less likely to have splenomegaly, basophilia, evidence of CML before or after treatment, and frequently show cytogenetic abnormalities such as loss of chromosome 7, a gain of chromosome 8 and complex karyotype [1,6,7,10]. The fusion transcripts p210 and p190 are generally equally distributed among *BCR-ABL*1+ AML patients [7,11]. Diagnostic challenges and controversy remain as to whether *de novo* AML with *BCR-ABL1* is a distinct entity and represents truly new leukemia or a transformed CML presenting in blast crisis [7,11].

The *RUNX1* gene, located on chromosome band 21q22, is a master regulator transcription factor, essential

for normal and malignant hematopoiesis [12]. Somatic and germline mutations of *RUNX1* may be seen in a variety of hematologic malignancies. Germline mutations of *RUNX1* may be associated with FPDMM [12]. *RUNX1* mutations have been identified in 5.6% of AML and associated with an inferior outcome [9].

RUNX1 mutations have recently been described in CML, including CML-BP [13] and in 38% of *BCR-ABL1*+ AML [14]. Thus, the presence of *RUNX1* mutation does not contribute to their distinction. The gene mutation profiles of CML-BP and *BCR-ABL1*+ AML show many similarities. Some of the most frequently mutated genes in *BCR-ABL1*+ AML are chromatin regulators *ASXL1*, *BCOR*, *BCORL1*, RNA splicing genes *SRSF2* and *SF3B1*, and *RUNX1* [14].

RUNX1 is one of the most commonly mutated genes in CML-BP with an incidence that varies from 12.9 to 33.3% [5,13]. Most of the mutations occur in the Runt homology domain, similar to that seen in AML. In a study conducted by Adnan Awad et al. on 59 CML patients, the most common recurrent somatic mutations identified in blast phase were *ABL1* (37%), chromatin regulators *ASXL1* (26%), *BCOR* (16%), and *RUNX1* (16%) a mutation profile similar to that observed in AML [15]. Downregulation of DNA repair genes such as CETN2, MLH1 and IKZF1 deletion has also been reported in RUNX1 mutated CML-BP (12).

Given the reported family history of hematologic malignancies and persistent bleeding experienced by our patient, the presence of *RUNX1* mutation raised the possibility of a germline familial condition such as FPDMM which is characterized by moderate thrombocytopenia ($75–140 \times 10^9$/L), bleeding and/or myeloid neoplasm with frequent strong anticipation among asymptomatic family members evident by younger age of onset of leukemia in these patients. Identifying patients with germline *RUNX1* mutation will help them benefit from genetic counseling, surveillance for the early detection of leukemic transformation, and screening of family member donors for allogeneic bone marrow transplantation.

In summary, the index patient presented with leukemia at an early age of 27 years without prior history of CML, leukocytosis, or basophilia. A complex karyotype harboring *BCR-ABL1* fusion in 100% of the metaphases and multiple copies of the *BCR-ABL1* fusion favored CML-BP. On the other hand, post-induction, there was a reversion of complex karyotype back to normal, with no *RUNX1* variant detected by sequencing accompanied by a decrease of the *BCR-ABL1* transcript p210 from >50% IS before induction to 0.282, and no evidence of CML post-therapy favoring a diagnosis of *BCR-ABL1*+ AML. At the time of preparing this report, the patient remains in remission, awaiting transplant.

While *BCR-ABL1* fusion underlies the pathogenesis of CML, it may not be sufficient to cause AML. The initiator event (class II mutation) is followed by a class I mutation that causes the leukemic transformation. It is speculated that the *RUNX1* mutation may represent the early event followed by the *BCR-ABL1* fusion that provides the proliferative advantage and development of the leukemic clone. Frequent observation of mutations in CML-BP as well as *de novo* AML, indicates that *RUNX1* mutations play an important role in leukemogenesis through hematopoietic stem cell upregulation and defective hematopoiesis.

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