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Concurrence of B-lymphoblastic leukemia and myeloproliferative neoplasm with copy neutral loss of heterozygosity at chromosome 1p harboring a *MPL* W515S mutation

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B-lymphoblastic leukemia (B-ALL) is a neoplasm of precursors committed to B-cell lineage, whereas myeloproliferative neoplasm (MPN) is a clonal proliferation derived from myeloid stem cells. Concurrent B-ALL with MPN is uncommon except in the presence of abnormalities of the *PDGFRA, PDGFRB, or FGFR1* genes or the *BCR-ABL1* fusion gene. Herein, we describe a rare concurrence, B-ALL with MPN without the aforementioned genetic aberrations, in a 64-year-old male patient. The patient was initially diagnosed with B-ALL with normal karyotype and responded well to aggressive chemotherapy but had sustained leukocytosis and splenomegaly. The posttreatment restaging bone marrow was free of B-ALL but remained hypercellular with myeloid predominance. Using a single nucleotide polymorphism microarray study, we identified a copy neutral loss of heterozygosity at the terminus of 1p in the bone marrow samples taken at diagnosis and again at remission, 49% and 100%, respectively. Several additional genetic abnormalities were present in the initial marrow sample but not in the remission marrow samples. Retrospective molecular studies detected a *MPL* W515S homozygous mutation in both the initial and remission marrows for B-ALL, at 30–40% and 80% dosage effect, respectively. In summary, we present a case of concurrent B-ALL and MPN and demonstrate a stepwise cytogenetic and molecular approach to the final diagnosis.

Keywords B-lymphoblastic leukemia/lymphoma/B-cell acute lymphoblastic leukemia, B-ALL, myeloproliferative neoplasm, copy neutral loss of heterozygosity, *MPL* mutation

B-lymphoblastic leukemia/lymphoma (B-ALL) is a clonal expansion of the immature B-cell precursors or lymphoblasts in bone marrow, blood, or lymph node. The incidence of B-ALL is approximately 1 to 4.75 per 100,000 individuals per year in the world (1). B-ALL occurs predominantly in the pediatric population, but it also occurs in adults with diverse cytogenetic and molecular background, most frequently in adults with a *BCR-ABL1* gene rearrangement, according to recent genetic data (2,3).

Myeloproliferative neoplasm (MPN) is another clonal hematopoietic stem cell neoplasm characterized by peripheral erythrocytosis, leukocytosis, or thrombocytosis, and bone marrow uni- or panlineage hyperplasia. Increased blast count can be seen in the accelerated or blastic phase of MPN (4). Between 2008 and 2010, the reported prevalence of MPN was shown to be approximately 5 to 57 per 100,000 persons per year (5). The BCR-ABL1 and JAK2 kinase pathways are known to be the drivers in the majority of MPNs. Whereas the *MPL* gene coding the thrombopoietic receptor regulates the proliferation of hematopoietic stem cells and megakaryocytes via activation of the JAK/STAT, ERK/MEK, and PI3K/

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AKT pathways, it also represents an alternative mechanism for MPN (6). Mutations of the *MPL* gene have been identified in 5% of primary myelofibrosis, 1% of essential thrombocythemia, and less than 1% of polycythemia vera cases but have never been reported in B-ALL (7). No data demonstrating the mutation have been found in any lymphoid neoplasm.

Concurrence of B-ALL and MPN is rare but could occur when there is an abnormality of the *PDGFRA, PDGFRB,* or *FGFR1* genes that is often accompanied by eosinophilia or in lymphoid blastic phase of chronic myeloid leukemia (2). There is limited information regarding driver genes involved in both myeloid and lymphoid differentiation or transdifferentiation (8,9). To our knowledge, B-ALL coexisting with MPN without any aforementioned gene aberration is extremely rare. There is also the risk that this concurrence may be underdiagnosed without the aid of advanced cytogenetic or molecular methodologies. In this report, we present an unusual case of B-ALL coexisting with MPN, describe our stepwise cytogenetic and molecular approaches to the final diagnosis, and discuss the possible underlying mechanisms.

Materials and methods

Clinical report

A 64-year-old Hispanic male presented with a weight loss of 25 pounds and moderate fatigue. He was sent to the emergency room, where it was noted that his white blood cell count was elevated to 51.3×10^9 cells/L, with a platelet count of 442×10^9 cells/L. Microscopic examination of his peripheral blood smear showed a normocytic anemia and left-shifted granulocytic maturation with 2% circulating blasts. No basophilia or eosinophilia was present. An imaging study by a CT scan exhibited splenomegaly, approximately 18.0 cm in the anterior–posterior dimension, and general lymphadenopathy involving the bilateral axillary lymph nodes, with the largest measuring 1.6×1.7 cm. A bone marrow biopsy showed 48% lymphoblasts in the aspirate (Figure 1A). Nearly 100% cellularity was demonstrated in the core biopsy, of which 50% was replaced by lymphoblasts and was associated with a background of atypical megakaryocytes (Figure 1B). Moderate reticulin fibrosis (2+) was noted in the bone marrow. A flow cytometric analysis revealed approximately 30% B-lymphoblasts expressing CD19, CD10, CD34, terminal deoxynucleotidyl transferase (TdT), CD13, and dim CD20. Immunohistochemical stains confirmed PAX5–positive and TdT-positive lymphoblasts (Figure 1C and D). The morphologic and phenotypic findings were diagnostic of B-ALL. Karyotyping, fluorescence in situ hybridization (FISH), and PCR studies for a *BCR-ABL1* gene rearrangement were performed to subclassify the leukemia and to guide clinical treatment. Single nucleotide polymorphism (SNP) microarray assay and additional molecular studies were performed when a concurrent hematopoietic neoplasm was suspected.

Cytogenetic analysis

FISH studies were performed using standard protocols (Cytogenetic Laboratory, Integrated Oncology-Laboratory Corporation of America, Winston-Salem, NC). Conventional G-banding cytogenetics studies had been performed in nonstimulated bone marrow aspirate from the patient at diagnosis and at remission of B-ALL following standard karyotyping procedures. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (10). FISH studies were performed using the 4q12 LSI PDGFRA, 5q32-q33 LSI PDGFRB, 8p12 LSI FGFR1, and 9q34 LSI ABL/22q11.2 LSI BCR probes for MPN (Abbott Laboratories, Abbott Park, IL).

SNP microarray analysis

SNP microarray analysis was performed using the Affymetrix Cytoscan HD platform (743,000 SNP/1,953,000 Non-Polymorphic Copy Number probes) (Santa Clara, CA). A total of 250 ng of genomic DNA was extracted and amplified with a GeneAmp PCR System 9700 (Applied Biosystems). PCR products were hybridized to the Affymetrix Cytoscan HD GeneChip, with data analyzed using Chromosome Analysis Suite (Cytogenetic Laboratory, Integrated Oncology-Laboratory Corporation of America). All breakpoints were based on build CRCh37/hg19 of the human genome reference sequence.

PCR analysis and sequencing

Reverse transcription–PCR (RT-PCR) was conducted in our in-house laboratory to identify the *BCR-ABL1* fusion transcript using the ABI Prism 7500 real-time PCR system (Applied Biosystems). PCR assays and the capillary gel electrophoresis method were used to test the clonal B-cell population. Total cellular DNA was extracted and amplified using BIOMED-2 primers, which targeted all three immunoglobulin heavy-chain frameworks and the immunoglobulin kappa light-chain (InVivoscribe Technologies, San Diego, CA). PCR studies for the *JAK2* mutation were performed by ARUP (Salt Lake City, UT), using an allele-specific primer that specifically initiates amplification from the allele containing the point mutation in codon 617. *MPL* (W515 and S505) mutation analyses were performed by Integrated Oncology-Laboratory Corporation of America, using Sanger sequencing covering exons 10 to 11 of the *MPL* gene. All of these tests were performed according to the standard procedures at each facility.

Results

At diagnosis of B-ALL, conventional cytogenetic analysis showed a normal male karyotype, 46,XY[20]. FISH and RT-PCR studies revealed no *BCR-ABL1* gene fusion products. The PCR study identified clonal immunoglobulin heavy-chain and light-chain kappa gene rearrangements.

The patient received hyper–central venous access device (CVAD) chemotherapy, including cyclophosphamide, vincristine, doxorubicin, cytarabine, methotrexate, and leucovorin followed by granulocyte-colony stimulating factor. Two months after completion of the last cycle of hyper-CVAD, arm 2B, his peripheral blood sample showed persistent leukocytosis (white blood count of 17.92×10^9