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A rare case of B-lymphoid blast phase of chronic myeloid leukemia: Diagnostic challenges

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ABSTRACT

Chronic myeloid leukemia(CML) is characterized by Philadelphia(Ph) chromosome. About 5% of cases are diagnosed in blast phase. We report a case of a 53-year-old female with no significant medical history, in B-lymphoblast crisis. Flow cytometry demonstrated B-lymphoblasts with no myeloid aberrancies, together with immature neutrophils in blood, and B-lymphoblasts in bone marrow. Cytogenetic studies identified Ph+ with complex abnormalities. Molecular analysis showed positive both for p210 and p190 transcripts in blood. ABL1 mutation analysis by Next Generation Sequencing(NGS) detected Thr315Ile mutation, which confers resistance to many tyrosine kinase inhibitors(TKIs). Eight months later she received allogeneic transplant and is doing well.

1. Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative hematopoietic stem cell disorder characterized by chromosomal translocation t(9;22)(q43.11;q11.2), resulting in formation of the Philadelphia (Ph) chromosome. The Ph chromosome is seen in 95% of patients with CML and in 15-20% of adult patients with acute lymphoblastic leukemia (ALL) [1]. Most cases of CML present in the chronic phase (CP). About 5% of cases are diagnosed in accelerated phase (AP) or blast phase (BP) without a recognized CP. In the majority of cases, blasts are of myeloid origin. Lymphoblasts are seen in 20-30% of BP [2]. The Ph+ ALL is defined by WHO classification as B-lymphoblastic leukemia with t(9;22)(q34.1;q11.2); BCR-ABL1. This type of B-ALL is thought to affect uncommitted progenitor cells, thus is more immature than other B-ALL case [3, 4]. CML is associated with the p210 protein transcript, while the p190 transcript is associated with ALL [2].

Here we report a patient with features of both CML and ALL with and laboratory findings of complex cytogenetics, B lymphoblasts, and presence of BCR-ABL1 p210 and p190 fusion transcripts. The various diagnostic approaches used to determine the etiology of her disease is discussed.

2. Materials and Methods

Standard cytogenetic G-band metaphase analysis and fluorescence in situ hybridization (FISH) were performed using standard techniques on peripheral blood (PB) and bone marrow (BM). G-banding was performed on 20 metaphase cells per analysis. FISH analysis was performed on 200 nuclei and/or 10 metaphase cells per probe.

FISHprobes: EGR1(5q31)/D5S23,D5S721, D7S486(7q31)/CEP7, CEP 8(D8Z1), BCR/ABL(22q11,9q34,), BCR(22q11), ABL1(9q34), KMT2A(MLL-11q23), p53(17p13.1),PML/RARA(15q22,17q21), 20q12 (D20S108). All probes were supplied by Abbott Molecular (Des Plaines, IL).

Flow cytometric analysis was performed using Beckman Coulter Navios 10-color flow cytometer and the data was analyzed with Kaluza Software version C (IVD). The antibody panels included ClearLLab 10C DURA clone reagents and add-on panels which were configured based on Children's Oncology Group (COG) B-ALL flow panels and EuroFlow acute leukemia orientation panel (see table 1).

The p210 and p190 BCR-ABL1 fusion transcripts were quantified by RT-qPCR. For the p190 analysis, RNA extracted from BM or PB (QiAamp RNA Blood Mini kit, Qiagen, MD) and reversed transcribed and amplified using the ipsogen FusionQuant kit (Qiagen, MD). The p190 results were expressed as % BCR-ABL1/ABL1 ratio. The p210 fusion transcript was quantified using X-pert BCR-ABL Ultra (Cepheid, CA). Results are expressed in the International Scale (IS). The upper LoQ is 10%. Next generation sequencing (NGS) was done on BCR-ABL1 genes on the Illumina NextGen sequencing platform (Illumina, CA).

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Table 1
Flow cytometry antibody panels

Panel	Markers (FITC/PE/ECD/PC5.5/PC7/APC/AF700/AF750/PB/KrO)									
Tube #1	Kappa	Lambda	CD10	CD5	CD200	CD34	CD38	CD20	CD19	CD45
Tube #2	TCRγδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8	CD3	CD45
Tube #3	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLADR	CD11b	CD45
Tube #4	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLADR	CD19	CD45
Tube #5	CD20	CD22	CD10	CD13+33	CD34	CD58	CD38	CD19	CD9	CD45
Tube #6	nTdT	cMPO	CD14	CD33	cCD22	cCD79a	CD19	CD34	cCD3	CD45
Tube #7	cMPO	CD135		CD33	CD56	CD34	CD11c		CD65	CD45

3. Results

A 53-year-old female, with past medical history of hypertension and smoking, presented to the emergency department with six days of worsening headaches. Her complete blood count showed total white cell count: 244.1 × 10^3/μL, hemoglobin: 5.2 g/dL, and platelet count: 180 × 10^3/ μL. The manual differential count reported 34% blasts, 40% neutrophils, 14% lymphocytes, 4% monocytes, 3% bands, 2% myelocytes, and 2% eosinophils.

Morphology: The PB smear showed hyperleukocytosis with leukoerythroblastosis, left shift and increase in blasts. The blasts contained deep blue cytoplasm without granules or Auer rods, prominent nucleoli, and oval to round nuclei.

The bone marrow aspirate (BMA) and touch prep were normocellular and showed a significant population of intermediate-size blasts. There were significant numbers of myeloid precursor cells, basophils, and eosinophils in the background, which raised the possibility of transformation from CML or myeloproliferative neoplasm. The BM biopsy was hypercellular with effacement of the marrow by blasts, which made up 80% of total marrow cells, as well as increased eosinophils and background fibrosis.

Flow cytometry: Immunophenotyping in PB demonstrated B-lymphoblasts (about 12% of WBCs), showing negative to dim CD45 expression, positive for CD19, CD10, CD34(variable), TdT, CD38(variable), HLADR, sCD22, cCD22, cCD79a, CD58, CD9; negative for sCD3, cCD3, surface light chain, MPO, and other myeloid/monocytic markers tested. A minor population of myeloid blasts (<1% of WBCs) was identified, expressing dim CD45, positive for CD34, CD33, CD117, MPO (variable), CD38, HLADR, and CD11c. Additionally, the myeloid cells presented full maturation pattern, which is usually seen in BM. The BMA showed an aberrant population of B-lymphoblasts (about 20% of WBCs), with the same immunophenotype as in the PB and no abnormal myeloid blasts were detected.

Cytogenetics: Chromosomal analysis revealed a complex karyotype with two abnormal related clones: 46~47,XX,+8,t(9;22)(q34;q11.2)[11]/45~46,XX,psudic(6;9)(q13;q34)(6qter→6q13::9q34→9p13),-7,+8,der(9)t(9;22)(9pcen→9p12::der(22)t(9;22)(q34;q11.2)(9qcen::der(9)t(9;?)(q22;?),+der(22)t(9;22)(q34;q11.2)x2[cp9]. The stem line (clone 1) had a karyotype of 46~47,XX,+8,t(9;22)(q34;q11.2). The sideline (clone 2) had a complex unbalanced rearrangement involving a dicentric (6;9)(q13;q34) translocation and an unbalanced derivative chromosome 9, der(9)(t(9;22)(q34;q11.2) on both the long arm and the short arm of the chromosome.

The FISH demonstrated monosomy 7 [61.5%], deleted 7(q31) [22.5%], and trisomy 8 [86.5%]. These abnormalities were consistent with the karyotype. The typical BCR/ABL1 rearrangement was seen in 47.5% of the nuclei and an additional fusion signals in 48% of the nuclei. This was consequent to the cytogenetic rearrangements.

Molecular genetics: The simultaneous presence of the p210 and p190 fusion transcripts was observed in both BM and PB. The p190 concentration in BM was 0.009% and 0.0016% in PB. The p210 concentration was >10% in both BM and PB. BCR-ABL1 mutation analysis by NGS detected a Thr315Ile mutation.

Based on these laboratory findings and the patient's clinical manifestation, the diagnoses was CML, BCR-ABL1-positive, in blast crisis with B-lymphoblasts.

4. Discussion

In the absence of a recognized chronic phase, differentiating CML-BP from Ph+ ALL can be a challenge. The diagnosis in this case of CML, BCR-ABL1-positive, in blast crisis with B-lymphoblasts rather than *de novo* precursor B-cell ALL was made based on a combination of findings, including blast morphology, immunophenotyping, chromosomal anomalies seen in advanced phase CML, and molecular findings.

In this case, flow cytometry immunophenotyping in PB and BMA demonstrated mostly B-lymphoblasts and myeloid cells presented features of CML. This patient had the characteristic t(9;22)(q34.1;q11.2) translocation. Blast crisis is related to the evolution of a Ph-positive clone and is accompanied by secondary chromosomal abnormalities, such as +8, +Ph, i(17q), +19, –Y, +21, +17, or monosomy 7 in 70–80% of cases [5]. The frequency of additional chromosomal abnormalities is around 5% in CP and increases to 50–80% in the AP [5]. This patient had monosomy 7 and trisomy 8, which are non-random abnormalities observed in myeloid disorders. Trisomy 8 and the additional Ph chromosomes are typically associated with CML [6, 7]. Trisomy 8 and extra copies of the Ph chromosomes can be seen in Ph+ALL with monosomy 9 with gains of 1q abnormalities [6]. This was not seen in our analysis.

The breakpoint in the majority of CML cases occurs in the major breakpoint cluster region (MBCR) with the M-BCR-ABL1 transcripts encoding a 210kD BCR-ABL1 fusion protein (p210). The minor breakpoint is more common in ALL, with mBCR-ABL1 transcripts encoding a smaller 190 kD BCR-ABL1 fusion protein (p190) [8]. The p190 transcript is rarely seen in CML (1-2% of patients). In the reported case, although the patient had no previous history or symptoms of CML, molecular genetic findings showed predominantly p210 (major) > 10% and a smaller (0.016%) p190 (minor) fusion protein. Upon reviewing all the laboratory findings, we determined the diagnosis of CML in lymphoid blast crisis.

In some cases, especially in the absence of documented chronic phase, CML in B-lymphoid blast crisis could resemble features of *de novo* Ph+ B-ALL, which makes the diagnosis challenging. However, in Ph+ B-ALL, BCR-ABL1 is restricted to lymphoblasts. A recent study by Chen *et al* suggested to detect BCR-ABL1 in the mature myeloid forms of bands and segmented cells under DAPI staining on FISH slides, which would indicate that myeloid cells are part of the Ph clone [9].

In general, CML-BP has a poor prognosis. The current standard treatment strategy for CML-BP utilizes tyrosine kinase inhibitors (TKIs), with or without induction chemotherapy based on the blast phenotype [1]. The goal is to revert the disease to CP and proceed to allogeneic stem cell transplantation as soon as possible. However, NGS analysis revealed the patient had a single amino acid substitution in the ABL kinase domain, Thr315Ile, which confers resistance to many TKIs including nilotinib, bosutinib, and dasatinib, but does not affect the use of ponatinib [10]. In a study by Nicholas Short and colleagues, the treatment combination of ponatinib and blinatumomab in newly diagnosed patients with chronic myeloid leukemia in lymphoid blast crisis (CML-LBC) or Ph chromosome–positive ALL resulted in a complete response rate of 100% and a complete molecular remission rate of 85% [11].

The hematology-oncology team decided upon a treatment plan with

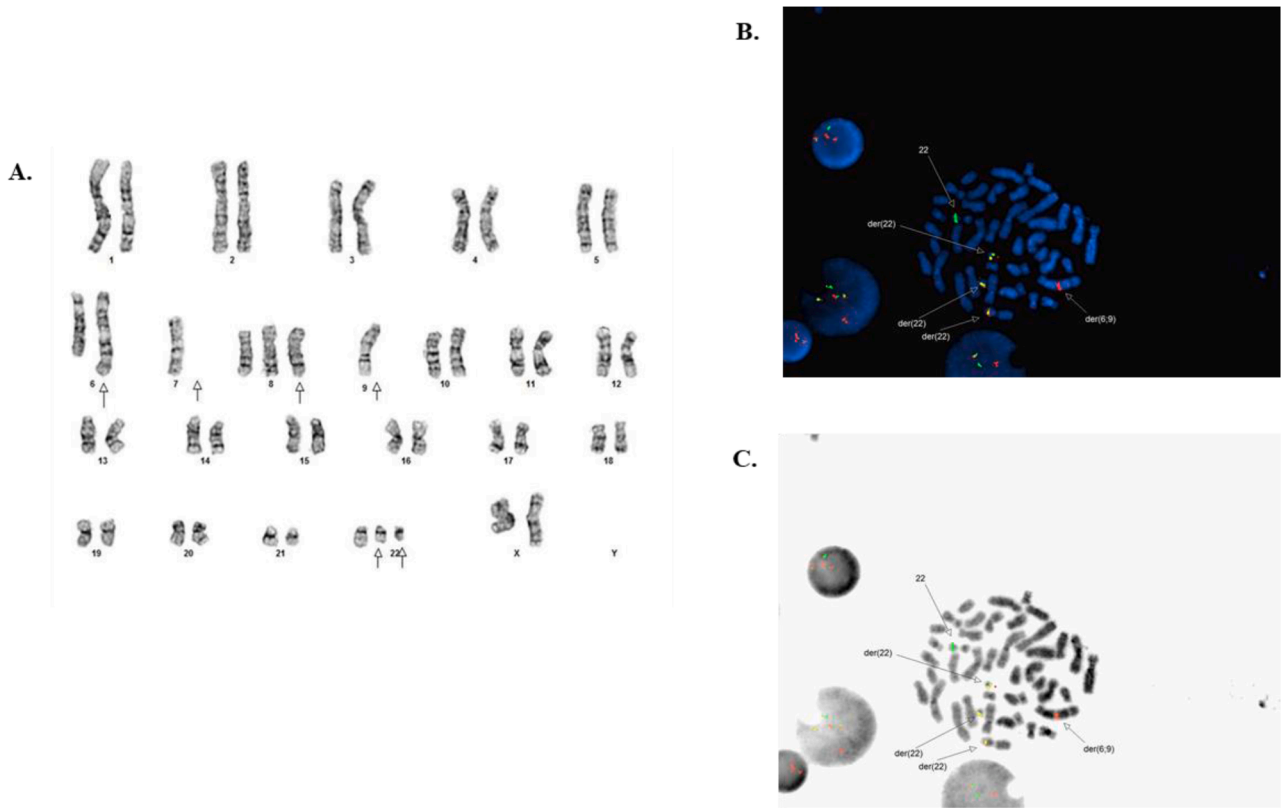

Fig. 1. A. Complex karyotype: Arrows indicate an unbalanced dicentric (6;9)(q13;q34) translocation,-7,+8, and an unbalanced der(9) derived from a t(9;22)(q34;q11.2) on both the long arm of the chromosome and on the short arm of the chromosome and 2 copies of the der(22)t(9;22)(q34;q11.2). B. DAPI image The red signal is the derivative chromosome 9, [derived from the dic(6;9)q13;q34)], the green is the normal chromosome 22. The 3 yellow signals at 3 o'clock are the der(22)t(9;22)(q34;q11.2)x3. C. Reverse DAPI image of the cell in B.

ponatinib and blinatumomab to achieve molecular response, followed by allogeneic stem cell transplantation.

Follow-up cases at four and six months, flow cytometry demonstrated no detectable B-lymphoblast, while cytogenetics were normal (46,XX). FISH showed minimal residual disease (MRD)with deleted 7q in 1% of the nuclei. The minor BCR-ABL1 fusion transcript p190 was negative and p210 positivity reduced to 0.0079%.

At eight months the patient received an allogenic hematopoietic stem cell transplant from her brother. Chromosomes showed all 46,XY (donor metaphases). Nuclear FISH showed 0.5% patient (XX) and 99.5% donor (XY), and 0.5% monosomy 7 and 1% deleted 7q31. These results were indicative of low level MRD. Both BCR-ABL1 p210 and p190 fusion transcript were not detected.

5. Conclusion

This case presented with a diagnostic dilemma as the patient had hematologic, molecular and cytogenetic features of both CML and ALL. The results of the above analyses were strongly indicative of a diagnosis of CML versus de novo ALL. These key factors were essential in determining the successful treatment plan for this patient. We are continuing to treat and closely monitor her. This case is an example of how complex patient presentations are diagnosed, prognosed, and treated. Additional studies are needed to rapidly and effectively treat these very ill patients. This case report did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.(Fig. 1)

Informed Consent

The informed consent was obtained by the referring physician.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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