A Chronic Myelogenous Leukemia (CML) Case with a Cryptic Insertion of the *ABL1* Gene of Chromosome 9 into 22 Resulting in a Fusion Signal on the Derivative 22: 46,XY.ish ins(22;9)(q11.2;q34q34)BCR+,ABL1+

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Abstract

Chronic myelogenous leukemia (CML) is characterized by the specific cytogenetic translocation t(9;22)(q34;q11.2), also called the Philadelphia (Ph) chromosome. We present a case of a cryptic *BCR/ABL1* fusion, which was not originally detected by standard karyotyping. The patient is a forty-seven-year-old man who presented with leukocytosis. Bone marrow biopsy was consistent with CML in chronic phase with no increase in myeloblasts. Conventional cytogenetic studies revealed a 46,XY karyotype. Despite this finding, the patient was started on hydroxyurea therapy followed by Gleevec. At six-month follow-up, a repeat karyotype was again normal, though FISH analysis was positive for *BCR/ABL1* fusion. FISH performed on previously G-banded metaphases showed a very rare cryptic insertion involving 22q11. While most genetic abnormalities in CML can be diagnosed using classical cytogenetics, molecular studies remain the gold standard in definitively identifying the characteristic *BCR/ALB1* fusion. This case represents one of the variant cryptic rearrangements in CML where clinical correlation with morphologic, immunophenotypic, cytogenetics and FISH findings are indicated and highlights the importance of molecular testing at the time of primary diagnosis.

Case Study

Chronic myelogenous leukemia (CML) is a hematological neoplasm that classically presents with hypercellular bone marrow, with myeloid preponderance, splenomegaly, granulocytosis, and thrombocytosis (Monma et al., 2004). The cytogenetic hallmark in CML is the Philadelphia chromosome (Ph) – present in up to 95 percent of cases (Monma et al., 2004). This results in a *BCR/ABL1* fusion gene with constitutive tyrosine kinase activity, which seems to be the critical factor in the leukemogenesis process. The remaining 5 percent of cases (Monma et al., 2004) show genetic variants of the Philadelphia chromosome involving a third chromosome. Around 1 percent of CML cases appear to have a Philadelphia negative karyotype but carry a cryptic *BCR/ABL1* fusion that can be identified by fluorescence in situ hybridization (FISH) (Monma et al., 2004).

Herein, we report a case with a very rare cryptic translocation which required FISH studies to detect the diagnostic cytogenetic abnormality that was missed by conventional chromosome studies. A 49-year-old male presented with splenomegaly, vertigo, and high WBC count (>700,000/mL). Bone marrow (BM) biopsy showed hypercellular marrow with marked myeloid preponderance. Flow cytometry detected a small population of myeloblasts (0.84%) with aberrant CD7 expression; granulocytes and monocytes showed partial CD56 expression. Karyotype revealed no cytogenetic abnormality. PCR testing was performed on peripheral blood and was positive for the *BCR/ABL1* fusion transcript with a ratio of 11.173, confirming the diagnosis of CML. Hydroxyurea and Gleevec therapy was given, and after six months, the WBC count had decreased to 53,000/mL. Repeat bone marrow biopsy showed persistent CML in chronic phase. Gleevec dose was increased, but soon reduced secondary to pancytopenia, which persisted, and the patient was switched to Sprycel. The most recent follow-up showed normalization of WBC counts (42,000/mL) with only mild anemia (Hb 9.6 g/dL) and pancytopenia (67 x 10^9/L).

Cytogenetic studies of the BM showed 46,XY[20] (Figure 1). FISH analysis was performed on interphase nuclei as well as in previously G-banded metaphases using the Vysis LSI *BCR/ABL* Dual color, Dual Fusion Translocation probe from Abbott Molecular (Des Plaines, Illinois 60018) on interphase nuclei and in previously G-banded metaphases. A fusion signal appeared in 195 (97.5%) of the 200 interphase nuclei analyzed (Figure 2A). Seventeen of the 195 cells (8.7% of the abnormal cells) also showed an extra green signal for the *BCR* gene (data not shown). FISH analysis on abnormal previously G-banded metaphases confirmed a cryptic translocation of the *ABL1* gene from chromosome 9 into chromosome 22, yielding a yellow fusion signal on chromosome 22 (Figure 2B). The results support a diagnosis

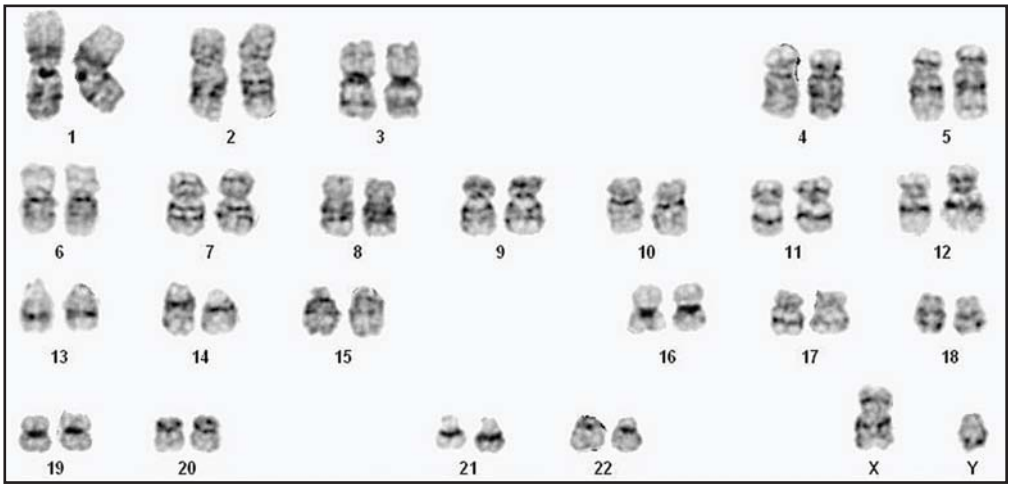

Figure 1. Karyotype of our patient: 46,XY.ish ins(22;9)(q11.2;q34q34)BCR+,ABL1+

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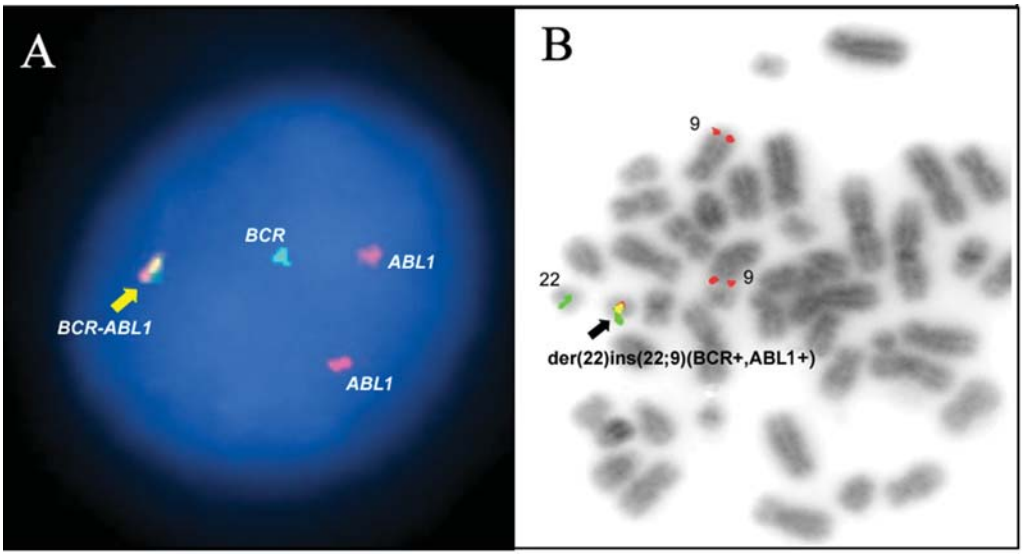

Figure 2A. Interphase FISH showing two *ABL1* signals on chromosome 9, a fusion *BCR/ABL1* signal and a *BCR* signal. **2B.** A previously G-banded metaphase showed a fusion signal on the Ph chromosome (arrow), and two *ABL1* signals on each copy of chromosome 9.

of a cryptic t(9;22)(34;11.2). These results were described as: nuc ish(ABL1x3,BCRx2)(ABL1 con BCRx1)[178/200]/(ABL1,BCR) x3(ABL1 con BCR1)[17/200].

The karyotype was modified to reflect the FISH findings, and reported according to the ISCN 2009 (Shaffer et al., 2009) as: 46,XY.ish ins(22;9)(q11.2;q34q34)(BCR+,ABL1+)

While 5 percent of CML patients present with Philadelphia chromosome variants, only 1 percent present with a normal karyotype (Testoni et al., 2009). Of that 1 percent, most cases show cryptic insertion at 22q11, as does this case. Only rarely is a cryptic insertion at 9q34 seen (Reddy and Grove, 1998; Wan et al., 2004; Valle et al., 2006; Virgili et al., 2008; Testoni et al., 2009).

Two mechanisms for this cryptic translocation t(9;22)(q34;q11.2) have been proposed (Reddy and Grove, 1998; Wan et al., 2004; Testoni et al., 2009). The first is a single step mechanism in which a small segment of 9q34 including the *ABL1* gene translocates to chromosome 22 and inserts proximal to the *BCR* gene at 22q11.2. Similarly, the reciprocal insertion of *BCR* into chromosome 9 proximal to the *ABL1* gene is also possible. The second method consists of two steps. After a classical t(9;22)(q34;q22.1), a second translocation returns the 9q and 22q arms to the original chromosomes, but the *ABL1* gene remains on chromosome 22 next to the *BCR* gene, or, alternatively, the *BCR* gene remains on chromosome 9 next to the *ABL1* gene. Both mechanisms of cryptic translocations have been described (Virgili et al., 2008). CML patients with these cryptic translocations seem to have similar clinical features and therapeutic response as those with a typical translocation (Monma et al., 2004). In the present study, it is possible that there was cryptic insertion of *ABL1* into chromosome 22 with loss of the derivative chromosome 9. However, the exact mechanism responsible for the normal karyotype cannot be determined. Regardless, treatment was not impacted.

The gold standard in CML diagnosis involves detection of *BCR/ABL1* fusion, which may require molecular testing. Our case illustrates the importance of a multidisciplinary approach including clinical, morphologic, immunophenotypic, cytogenetic, and molecular correlation.

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