**Clinical Report**

**Saudi Arabian CML patient with a novel four-way translocation at t(9;22;5;2)(q34;q11.2;p13;q44)**

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**Abstract**

**Background:** The vast majority of chronic myeloid leukemia (CML) patients have a single translocation t(9;22)(q34;q11), BCR/ABL1 fusion genes, which is regarded as the hallmark of CML. However, around 5 to 10% of CML patients exhibit the involvement of a third chromosome. In some very rare cases a fourth or even fifth chromosome can be involved with the t(9;22).

**Methods:** This case report is based on a 40-year-old Saudi Arabian male patient, diagnosed with CML in lymphoid blast crisis, and observed to have a four-way 46 XY, t(9;22;5;2)(q34;q11.2;p13;q44) translocation. The BCR/ABL1 fusion was identified by fluorescent in situ hybridization (FISH). Additionally, the BCR/ABL1 p210 mRNA fusion transcripts were identified by a molecular test.

**Results:** The clinical and prognostic impact of additional partner chromosomes to t(9;22) remains unknown. The CML patient with this novel four-way translocation t(9;22;5;2) progressed to blast crisis and was resistant to Tyrosine Kinase Inhibitor (TKI) therapy. Therefore, this case is more in alignment with the negative impact of additional partner chromosomes to the translocation at t(9;22).

**Conclusion:** Here we report for the first time a novel four-way translocation at t(9;22;5;2)(q34;q11.2;p13;q44).

**Keywords**

BCR/ABL1 fusion genes, chronic myeloid leukemia (CML), complex translocation, lymphoid blast crisis

**1 | Introduction**

Chronic myeloid leukemia (CML) is characterized by the unrestricted growth of pluripotent myeloid lineage cells due to a single reciprocal translocation t(9;22)(q34;q11) (Rowley, 1973; Thompson et al.,). This chromosomal translocation is characteristic of the disorder and results in the formation of the disease's hallmark, the Philadelphia (Ph) chromosome with the ABL proto-oncogene from chromosome 9 fusing onto the breakpoint cluster region (BCR) locus on chromosome 22 (Naumann & Decker, 2003; Nowell & Hungerford, 1960; Sherbenou & Druker, 2007). The fusion within the Philadelphia chromosome generates an oncogenic chimeric tyrosine kinase...
BCR/ABL1, whose activity is critical for the development of the leukemia phenotype of CML cells, via the unregulated phosphorylation and activation of intracellular signaling proteins that are responsible for the survival and growth of progenitor cells in the bone marrow (Zhou & Xu, 2015). While the translocation clearly keeps the tyrosine kinase signaling pathway active, the cause and mechanism of the translocation within the proliferated cells are still unknown (Rowley, 1973). Moreover, in 5 to 10% of CML patients, an additional chromosome is involved in the translocation with the t(9;22) region (Naumann & Decker, 2003; Thompson et al.). In 2019, a case was reported including a new variant pH translocation involving three chromosomal aberrations 6p22, 9q34, 22q11.2 and derivative chr 19 not described previously (Ciftciler et al., 2019). In 2012, a summary of 10 previously reported cases with five-way translocations of CML was reported (Yokota et al., 2012). In some very rare cases, a fourth chromosome can be involved (Asif et al. (2016); Kim et al. (2015); Kubota and Waki (2010)). Finally, in exceptional cases, a fifth chromosome can be involved with t(9;22) (Ikuta et al., 2008; Vaidya et al., 2013; Yokota et al. (2012)). This case report demonstrates a novel four-way translocation t(9;22;5;2) (q34;q11.2;p13;q44) uncovered in a 40-year-old Saudi Arabian male patient. The patient was diagnosed with CML in lymphoid blast crisis.

2 | CASE REPORT

A previously healthy 40-year-old Saudi male presented with generalized pain mainly chest and lower limb pain for a duration of one week. Four months prior to presentation, the patient went for blood donation and he was rejected because of high white blood cell (WBC) count, which was not investigated further. The patient was otherwise healthy and not on any medication. There were no other constitutional symptoms. At the time of presentation, physical examination was remarkable for skin rash over his chest, tenderness over the ribs, and tenderness over the anterior part of the right thigh with a cord like sensation, there was no hepatosplenomegaly.

Laboratory evaluation showed complete blood count revealing leukocytosis (68 × 10^9/L), thrombocytopenia (10^5 × 10^9/L) and hemoglobin was 12 g/dL. The Peripheral blood film showed: 54% blast cells, 30% Lymphocytes, 11% neutrophils, 2% promyelocytes, 2% myelocytes, 5% eosinophils, and 2% basophils. The bone marrow studies showed a hyper cellular marrow with 80% of blasts with deep purple granules in the May–Grunwald–Giemsa stain: 2% erythroid precursor cells, 2% promyelocytes, 2% myelocytes, 3% lymphocytes, 5% eosinophils, and 2% basophils. The flow cytometry showed increase in both eosinophil and basophil precursor cells with residual myeloid precursor cells. The cytogenetic analysis identified the following karyotype: 46,XY, t(9;22;5;2) (q34, q11.2, q13, q44) in 98% of analyzed metaphases. The molecular analysis revealed a 47% expression level of BCR/ABL1 p210 mRNA transcripts.

The diagnosis of the patient was lymphoid blast crisis CML. The flow cytometry support was based on the presence of leukocytosis with increase in eosinophil, basophil and myeloid precursors. Patient was started on the Tyrosine Kinase Inhibitor (TKI), Dasatinib 140 mg daily, and hyper CVAD protocol (hyper fractionated cyclophosphamide; vincristine; doxorubicin; and dexamethasone; alternating with high dose methotrexate and cytarabine). Intrathecal chemotherapy administration was added to the protocol. The patient achieved complete remission from a hematological standpoint and his BCR/ABL1 went down from 47% to 1.5% after the first cycle of treatment. He completed the chemotherapy as per protocol and achieved molecular remission with minimal residual disease (MRD) negativity. Based on the lymphoid blast crisis CML high risk disease, the patient was admitted for allogenic stem cell transplant from a haploidentical sibling donor (brother). His Conditioning regimen included Fludarabine, Cyclophosphamide and total body irradiation (TBI) with post-transplant cyclophosphamide. Skin graft versus host disease (GVHD) prophylaxis included Tacrolimus and mycophenolate-mofetil (MMF). The patient suffered from multiple complications post-transplant including skin GVHD that responded to steroids; Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivation; and chest fungal infections. During the first few months, his engraftment was successful and he achieved 100% chimerism status. BCR/ABL1 p210 mRNA transcripts were detectable at low levels at 30 days post-transplant (0.0007%) and increased further at 60 days at (0.011%) for which immunosuppressant medications were adjusted and TKI reintroduced. He then achieved molecular negativity. Shortly after that, we detected a regain in the proliferation activity of the abnormal tumoral clone passing from 0.011% to 0.026% p210 mRNA transcripts and the patient passed the way after developing a graft versus host disease.

3 | CYTOGENETICS

The conventional chromosomal analysis showed 46XY with t(9;22;5;2)(q34;q11.2;p13;q44) translocation in 16 analyzed metaphases (Figure 1). There are two possible major mechanisms of this translocation. The first one, t(9;22) occurs as first event and thereafter, chromosomes 5 and 2 become implicated in the process as second event.
The second mechanism can involve all four chromosomes, 9, 22, 5, and 2 from the beginning of the translocation process. The molecular cytogenetics by FISH analysis showed the presence of complex BCR/ABL1 fusion genes with only one signal of fusion genes in 98% of analyzed nuclei (Figure 2). These results are in concordance with the implication of chromosomes 2 and 5 as third and fourth partners, respectively, in the t(9;22) rearrangement. The p210 BCR/ABL1 mRNA transcript (b2a2) was detected through standard molecular analysis; BCR/ABL1 imatinib test resistance was not performed. Unfortunately, the Spectral karyotyping and metaphase FISH are not available at our institution. Also, our Send Out testing is locked by our institution.

In general, the clinical and prognostic impact of t(9;22) with a third variant chromosome, t(9;22,v), is variable. By comparing TKI response and disease progression in CML patient with t(9;22) and CML patient with t(9;22,v), Aliano et al. found that the presence of t(9;22;v) in CML patient is associated with greater resistance to imatinib, transformation to blast phase and karyotype evolution (Stefania Aliano et al., 2013) (Aliano et al., 2013). Also in the same perspective, reported a complex case of three-way t(6;9;22) with a new variant. They concluded by associating their complex case to a poor prognosis. Different Physio-pathological processes can explain to some extent the non-favorable course, one such being that the third additional chromosome variant is associated with an increased number of chromosomal abnormalities which leads to genetics instability. Such genetics instability may result in treatment resistance (Kanakasetty et al., 2017). Another possibility is that during the translocation process there is loss of genetic material. These deletions can include some tumor suppressor genes and can result in enhanced tumor clone aggressiveness. However, other authors did not find any statistical difference by comparing the two CML groups (Kanakasetty et al., 2017). The clinical and prognostic impact of these additional third and fourth partners to t(9;22) in CML patient mentioned in this case is most likely associated with greater TKI
To protect patient's privacy, the data is available under request.

FIGURE 2 Dual-fusion FISH on interphase nuclei confirming the chromosomal analysis results by showing the involvement of chromosomes 2, 5, 9 and 22 in the translocations process in a CML patient with 46XY and t(9;22;5;2)(q34;q11.2;p13;q44) four-way translocation. Green probe corresponds to the BCR locus on chromosome 22 and Orange probe corresponds to the ABL1 locus on chromosome 9. The yellow fusion signal is indicative of the BCR/ABL1 and ABL1/BCR reciprocal translocation (t(9;22)) resistance and transformation to blast phase. The patient evolution over time at mid and long term will likely define more precisely if the added translocation complexity will confer negative impact. Finally, more cases are needed to support this evidence and more investigation is needed to define the clear state of the ongoing biological changes induced by a four-way translocation process at t(9;22).

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS
W. D., A. M., Y. L., P. S., and H. H. conceived the study and edited and coordinated the manuscript. The manuscript was drafted by S. K., H. R., W. D., and A. M., who organized data contents, reviewed literature, and completed figures and tables. W. D., H. H., and H. R. interpreted the molecular data. All authors contributed to the writing and reviewing of the manuscript and approved its final version.

EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS
The study was approved by ethics committee of King Fahad Specialist Hospital-Dammam, Saudi Arabia.

DATA AVAILABILITY STATEMENT
To protect patient's privacy, the data is available under request.

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