Abstract. Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder. It is characterized by the presence of the Philadelphia (Ph) chromosome, t(9;22)(q34.1;q11.2), which carries the BCR-ABL1 fusion gene. Tyrosine kinase inhibitors (TKIs) have markedly changed the treatment approach of CML and have become the first-line agents for almost all CML patients. However, certain patients experience resistance to these medications, which occurs through several mechanisms, including the accumulation of TKI-resistant chromosomal abnormalities. The present study reports a case of a 27-year-old Saudi male with CML receiving TKI treatment, who presented with precursor B-cell lymphoblastic crisis demonstrating the presence of the novel combined chromosomal abnormalities; non-Ph der(22), i(9) and der(20), carrying the BCR-ABL1 fusion gene. This case report adds to the literature on novel TKI-resistance-conferring chromosomal abnormalities and links them to precursor B-cell lymphoblastic crisis.

Introduction

CML is a myeloproliferative disorder that is characterized by the presence of the reciprocal translocation t(9;22), which forms the Philadelphia (Ph) chromosome. During this translocation, the breakpoint cluster region (BCR) gene at position 22q11.2 is juxtaposed to the c-Abelson (ABL1) gene at 9q34.1, forming the BCR-ABL1 fusion gene, which encodes a constitutively active tyrosine kinase (TK) protein (1,2). The constitutively active protein is associated with increased levels of erythrocytes, monocytes, megakaryocytes, myelocytes and platelets in the peripheral blood and marked myeloid hyperplasia in the bone marrow (3).

CML presents in one of three phases: Chronic phase, accelerated phase or blast crisis. The latter is of myeloid, lymphoid or mixed-lineage phenotype (4).

TKIs have markedly changed the approach to CML management. TKIs have improved patient outcomes to the extent that they are now currently accepted as the first-line agents for nearly all patients with CML, regardless of the phase of the disease. However, certain patients experience resistance to these medications; this occurs through several mechanisms including the accumulation of additional cytogenetic abnormalities, which can confer a survival advantage to the treated myeloid cells. The most common cytogenetic abnormalities include an additional Ph chromosome, trisomy 8 and isochromosome 17q (5,6). Several other less common cytogenetic abnormalities have been reported; however, to the best of our knowledge, those found in the present case have not been previously reported.

Case report

Presentation. The present case involves a 27-year-old Saudi male patient, whose initial presentation was three years prior. At that time, he presented with pallor and abdominal distension. He was revealed to have significant splenomegaly and marked leukocytosis, with a white blood cell (WBC) count of 105x10^9/l. Subsequent investigations confirmed the diagnosis of CML. The patient was initially treated with imatinib; however, due to myelosuppression, the treatment was changed to dasatinib. Subsequently, due to a skin reaction, the treatment was changed to nilotinib (100 mg/day), which the patient clinically responded to and tolerated well.

Keywords: chronic myeloid leukemia, precursor B cell, blast crisis, tyrosine kinase inhibitor, cytogenetic abnormalities
At his current presentation, the patient had fever, bone pain and cytopenia. Investigations confirmed the diagnosis of precursor B cell acute lymphoblastic leukemia with the presence of the novel combined chromosomal abnormalities of non-Ph der(22), i(9), and der(20), carrying the BCR-ABL1 fusion gene.

Complete blood count (CBC). CBC with differential was performed using an Automatic Hematological Analyzer Sysmex XE-5000 (Sysmex America, Inc., Lincolnshire, IL, USA).

Immunophenotyping. Immunophenotyping was performed on the patient's bone marrow aspirate as follows; upon collection of the bone marrow aspirate, 0.5 ml of the sample was mixed with 10 ml of red blood cell lysing solution and centrifuged at 540 x g for 5 min. The supernatant was discarded and the cell pellet further washed with PBS. A 100 µl aliquot of cell suspension with an adjusted concentration of 10x10^6 cells/l was added to tubes containing commercial pre-titrated volumes of labelled monoclonal antibody cocktails to bind several antigens, surface and cytoplasmic clusters of differentiation (CD) (BD Biosciences, San Jose, CA, USA) and incubated in the dark for 15 min at room temperature. These monoclonal antibodies were used in conjunction with four fluorochromes [fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) and peridinin chlorophyll (PerCP)] in each tube where they were diluted by factor of 20. FITC labelled antibodies bound to CD14 (cat. no. 561712), surface and cytoplasmic IgG (cat. no. 560952), surface and cytoplasmic CD3 (cat. no. 561806), CD34 (cat. no. 560942), cytoplasmic CD66 (cat. no. 551479), IgM (cat. no. 562029), CD33 (cat. no. 561818), CD38 (cat. no. 560982), CD2 (cat. no. 561759), CD64 (cat. no. 560970), terminal deoxynucleotidyl transferase (TdT) (cat. no. 347194), CD45 (cat. no. 560976) and (myeloperoxidase) MPO (cat. no. 340580). PE labelled antibodies bound to surface and cytoplasmic IgG (cat. no. 560951), cytoplasmic CD11 (cat. no. 560999), CD8 (cat. no. 560959), CD10 (cat. no. 561002), CD56 (cat. no. 561903), CD117 (cat. no. 561682), CD13 (cat. no. 561934), CD58 (cat. no. 560959) and cytoplasmic CD79a (cat. no. 555935). APC labelled antibodies bound surface and cytoplasmic IgG (cat. no. 562025), CD20 (cat. no. 560900), CD4 (cat. no. 561840), CD19 (cat. no. 561742), CD15 (cat. no. 561716), CD22 (cat. no. 562860), CD11b (cat. no. 340937), CD5 (cat. no. 340583), HLA-DR (cat. no. 560896), cytoplasmic CD22 (cat. no. 562860), CD25 (cat. no. 340939) and CD34 (cat. no. 560940). PerCP labelled antibodies bound CD45 (cat. no. 561086) and cytoplasmic CD3 (cat. no. 347344).

For intracellular staining (i.e., for CD79a, CD3, TdT and MPO), lymphocyte permeabilization preceded the addition of cytoplasmic and nuclear antibodies. This was achieved by adding 0.5 ml FACS Permeabilizing Solution to 100 µl of the lysed sample followed by 10-minute dark incubation at room temperature. The FACS Permeabilizing Solution was prepared by diluting 1 ml of BD Permeabilizing stock solution (cat. no. 340973; BD Biosciences) in 9 ml of distilled water.

The samples were processed with a FACSCanto II cell analyzer and the analysis was performed using FACS Diva Software (version 8.0.1; BD Biosciences). The flow cytometry data was analyzed with a threshold of 25,000 events. For gating, forward scatter, side scatter, CD45, CD3, CD19 in addition to other lineage specific markers were used. The lineages of the blasts were determined in each case depending upon the expression of lineage-specific markers where an expression for a certain marker was considered positive if the percentage of the cells expressing that marker was ≥20% and negative if <20%, except for TdT and MPO where the threshold was 10% as recommended (7).

Cytogenetic analysis. Chromosomal analysis using GTG banding was performed as described previously (8). Karyotyping was performed in 20 metaphases from the patient's unstimulated bone marrow sample according to the nomenclature of the International System for Human Cytogenetics (9).

Fluorescence in situ hybridization (FISH). FISH was performed using Vysis BCR-ABL1 Tri-Color Dual-Fusion FISH Probes (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA) to detect the BCR-ABL1 translocation, as described previously (10).

Initial presentation. At initial presentation, the patient's CBC with differential revealed a hemoglobin (Hb) level of 8.6 g/dl, a platelet count of 188x10^11/l and a WBC count of 105x10^9/l, comprised of 52% neutrophils, 1% lymphocytes, 3% monocytes, 0% eosinophils, 0% basophils, 1% promyelocytes, 2% myelocytes and 5% blast cells. The patient's peripheral blood smear revealed normocytic hypochromic anemia with anisocytosis and schistocytosis. There was marked leukocytosis with a significant number of immature myeloid precursors, indicating leukoerythroblastosis. The patient's bone marrow aspirate was hemodiluted, but revealed moderate cellularity with the presence of myeloid, erythroid and megakaryocytic lineages and ~5% blast cells. The karyotype of each of the 10 metaphases obtained from the bone marrow aspirate was 46,XY,t(9;22)(q34.1;q11.2). Karyotyping was performed in 20 metaphases from the patient's unstimulated bone marrow sample according to the nomenclature of the International System for Human Cytogenetics (9).

Molecular analysis. EDTA whole blood samples from the patient were used for quantification of the BCR-ABL1 P210 transcript by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). This was performed using the GeneXpert® Dx System (Roche Diagnostics GmbH, Mannheim, Germany) as described previously (11).

Current status. At the current presentation, the patient's CBC with differential revealed an Hb level of 10.5 g/dl, a platelet count of 37x10^11/l and a WBC count of 0.9x10^9/l, comprised of 48% neutrophils, 25% lymphocytes, 6% variant lymphocytes, 2% monocytes, 0% eosinophils, 0% basophils and 14% blast cells. The peripheral blood smear revealed pancytopenia with the presence of blasts.
Figure 1. (A) Giemsa-banding karyogram of the patient's bone marrow. Isochromosome 9q, derivative chromosomes 20 and 22 (red arrows) are presented, where the extra material on 20 containing BCR-ABL1 fusion is derived from chromosome 22. (B) Ideogram of all chromosomal abnormalities observed in this case with breakpoints marked.

Figure 2. FISH analysis of one metaphase and one interphase nucleus. (A) Showing two ABL1 (red) signals, one BCR signal (green), and two fusion gene BCR-ABL1 signals (yellow). (B) Labelled inverted image showing two ABL1 signals on the two long arms of isochromosome 9 (red), one BCR signal (green) on chromosome 22, and two fusion gene BCR-ABL1 signals (yellow) one on the long arm of der 9 and one on that of der 20, confirming the abnormalities seen on the karyogram. (C) Non-labelled inverted image identifying the chromosomes containing the signals.
The bone marrow aspirate revealed markedly increased blasts with markedly decreased myeloid, erythroid and megakaryocytic cell lineages. Immunophenotyping analysis of the aspirate revealed that the blast cells were positive for CD34 (partial, i.e., only a subset of the population of interest was positive), cytoplasmic CD79a, CD19, CD10, cytoplasmic TdT, CD20, HLA-DR, CD73 (partial), CD58, CD44, CD200, CD24, cytoplasmic CD66 and CD72 antigens. The cells were negative for cytoplasmic myeloperoxidase, cytoplasmic CD3, surface CD3, CD7, surface IgM, CD45 (negative to low), CD117, cytoplasmic CD22, CD33, CD13, CD38, CD123 and CD86 antigens, consistent with the precursor B-cell lymphoblastic nature of these blast cells.

A bone marrow biopsy revealed 99% cellularity with 95-99% blasts. The number of morphologically normal myeloid, erythroid and megakaryocytic cells was markedly decreased to absent. Karyotyping performed on 20 metaphases from the bone marrow revealed the following: 46,XY,i(9)(q10),der(22)t(9;22)(q34.1;q11.2)t(20;22)(q11.2;q11.2)[18]/46,XY[2] (Fig. 1A). This result, combined with that of the FISH analysis, confirmed the presence of a clone with the concurrent cytogenetic abnormalities of i(9)(q10), non-Philadelphia der(22) and der(20) carrying the BCR-ABL1 fusion gene (Figs. 1B and 2A-C).

**Discussion**

The blast crisis demonstrated in this case was of the precursor B cell lymphoblastic type. It is well established that ~30% of blast crises in CML are of the lymphoid rather than the

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**Figure 3.** Ideogram illustrating the formation of the cytogenetic abnormalities observed in this case. (A) Ideogram of the proposed first step where a reciprocal translocation between chromosomes 9 and 22 (red arrows) formed the Philadelphia (Ph) and derivative (der) 9 chromosomes. (B) Ideogram of the proposed second step where a reciprocal translocation (bidirectional red arrow) between chromosome 20 and Ph chromosome formed der(22) and der(20) chromosomes (the latter carrying the BCR-ABL1 fusion gene). (C) Isochromosome 9q [i(9q)] formation as an additional cytogenetic abnormality that did not necessarily occur in this order.
myeloid phenotype. The aberrant cytogenetic abnormalities observed in this case, in addition to the **BCR-ABL1** fusion during Ph chromosome formation, were i(9q)(q10) formation and the reciprocal translocation between the Ph chromosome and 20q11.2. To the best of our knowledge, this is the first case reported to combine these cytogenetic aberrations. This case report also links these cytogenetic aberrations to the precursor B cell lymphoblast phenotype. Ascertainment of the blast phenotype has its own therapeutic implications, since the treatment protocol of lymphoid blast crisis is different to that of the myeloid type.

In conclusion, the present study reports a case of TKI-resistant Ph-positive-CML presenting with lymphoblastic crisis wherein the blast cells, in addition to the Ph chromosome, exhibited additional novel combined cytogenetic abnormalities. This report adds to the literature on newly identified TKI-resistance-conferring cytogenetic abnormalities, and also links them to precursor B cell lymphoblastic crisis. This also has its own therapeutic implications since the blast phenotype determines the treatment protocol.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

HAK wrote the manuscript, drew the figures, designed the study, interpreted the data, contributed to addressing all questions related to the accuracy and integrity of this study, and initiated this research project and interpreted the cytogenetic results. AA followed up the patient and provided the test results. All authors read and approved the final manuscript.

Ethics approval

Approval from Johns Hopkins Aramco Healthcare (JHAH) Institutional Review Board and Ethics Committee was obtained to publish this case report.

Patient consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying figures.

Competing interests

The authors declare that they have no competing interests.

References


