CASE REPORT

A rare *t* (9; 12; 22) (q34; q23; q11) translocation in a patient with typical chronic myeloid leukemia: A case report

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Abstract

Chronic myeloid leukaemia (CML) is typically associated with reciprocal translocation between long arms of chromosome 9 and 22, *t* (9, 22) (q34; q11.2) and with the formation of a *BCR-ABL* fusion gene. In a minority of newly diagnosed CML cases, complex cytogenetic variants of the Ph chromosome can be observed with involvement of chromosomes 9, 22 and a third chromosome. Herein, we describe a lady with CML in chronic phase with a complex translocation involving chromosomes 9, 22 and 12. Conventional karyotyping revealed *t* (9, 12). Fluorescence in-situ hybridization (FISH) showed *BCR-ABL* fusion signals in 60% nucleated cells (cut-off levels ≥5% for positive signals). Whole chromosome painting (WCP) showed presence of a complex variant translocation between chromosomes 9, 12 and 22. However, DNA analysis for *BCR-ABL* fusion gene was negative. Cytoreductive therapy and Imatinib treatment were initiated.

Key words

Chronic myeloid leukaemia, Complex variant translocations, Philadelphia chromosome, *BCR-ABL*

1 Introduction

Chronic myeloid leukaemia (CML) is characterized by the reciprocal translocation of chromosome 9 and 22, resulting in formation of the *BCR-ABL* fusion gene on chromosome 22, which is also known as Philadelphia chromosome (Ph) [1]. An abnormal tyrosine kinase protein is produced from this *BCR-ABL* gene and plays a pathogenetic role in the leukemogenesis of CML and its clinical course [2]. The oncogenic protein tyrosine kinase, which is located in the cytoplasm, is responsible for the leukemia phenotype through the constitutive activation of multiple signaling pathways involved in the cell cycle and in adhesion and apoptosis [3].

There are 5-10% of CML cases noted to have variant Ph translocations and these findings have been reported since the past 20 years [4, 5]. Simple variants are cases that involved chromosome 22 with a chromosome other than 9, and complex variants are cases that involved chromosomes 9, 22 and one or more other chromosomes [6]. In complex variant translocations (CVTs), chromosomes other than 22 or 9 have been reported to act as the third chromosome [7, 8]. However, only a few cases with the long arm of chromosome 12 involvements have been reported so far. Occasionally, the chromosome changes are submicroscopic so the translocation can be masked and revealed only by molecular analysis or by fluorescence in-situ hybridization (FISH) [3]. Despite being genetically intricate in nature, available data indicate that CVTs at diagnosis do not represent a negative prognostic factor for CML patients [9, 10]. However such cytogenetic changes could be viewed as the expression of an underlying genomic instability, which is credited for the progression of the disease towards the accelerated and acute phase [11, 12]. It has been previously suggested that the Ph chromosome itself may arise from the unfaithful repair of DNA double stranded breaks, and such events may contribute to the occurrence of additional nonrandom chromosomal abnormalities [11]. Data from another recent study suggest that CVTs involving the Ph chromosome are associated with a more aggressive form of CML, thus conferring an unfavourable clinical outcome [12].

We report a case of typical CML in chronic phase with complex variant translocations involving chromosomes 9, 22 and 12; and discuss the clinical relevance of complex translocations in patients with CML.

2 Case report

A 55-year-old Malay lady was referred from a private hospital with one month history of progressive abdominal distension and discomfort associated with intermittent low grade fever, lethargy, worsening appetite and intermittent headaches. In 1998, she had a right mastectomy to remove a breast carcinoma. The patient underwent no adjuvant chemotherapy or radiotherapy and she had no medical follow up after the surgery. On examination, she had mild pallor and fever with a temperature of 38.4℃. The patient presented with massive hepatosplenomegaly and mild pedal oedema, but no lymphadenopathy was noted.

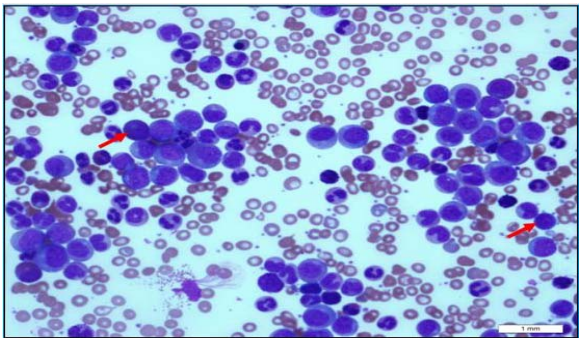

Figure 1. Peripheral blood film showed leukoerythroblastosis, hyperleukocytosis exhibiting all stages of granulocytic maturation with bipeak populations of segmented neutrophils and myelocytes, mild eosinophila and 2 % blasts. (Wright stain × 400). The red arrows point out examples of blasts observed in the peripheral blood.

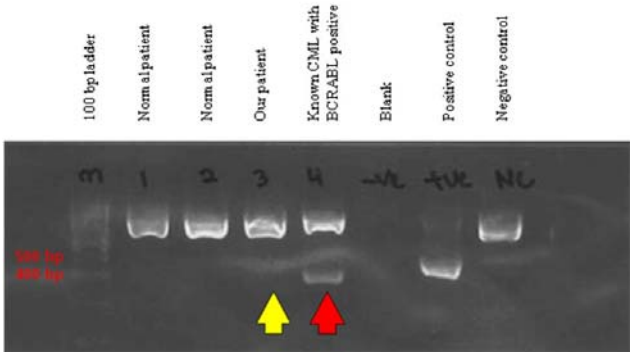

Figure 2. DNA analysis of marrow sample showed that the patient (yellow arrow) was negative for *BCR-ABL* fusion gene. Red arrow was a positive *BCR-ABL* control from a known patient (385bp).

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Laboratory investigations revealed low haemoglobin (8.4 g/dL), hyperleukocytosis (466 × 10^9/L) and mild thrombocytosis. Peripheral blood film showed leukoerythroblastosis with all stages of granulocytic maturation seen with a bipeak of segmented neutrophils and myelocytes and 2% blast cells (Figure 1). Renal profile was unremarkable, serum lactate dehydrogenase was elevated (3155 U/L) and her neutrophil alkaline phosphatase (NAP) score was low (16/100 neutrophils). Bone marrow aspirate smear showed findings that were similar with her peripheral blood film. Trephine biopsy showed hypercellular marrow with myeloid series proliferation which displayed all stages of maturation with decreased erythroid series. Her calculated Sokal score was 1.75 (high risk).

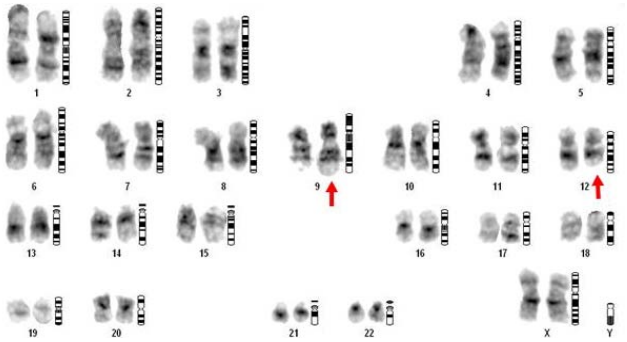

Figure 3. Chromosome analysis of patient's blood sample demonstrated female chromosome (46 XX) with translocation between long arm of chromosome 9 and 12, *t* (9; 12)(q34;q23) (red arrows).

Molecular analysis showed that this patient was negative for the *BCR-ABL* fusion gene (Figure 2). Chromosome analysis showed presence of translocation between chromosome 9 and 12 (Figure 3). Fluorescent in-situ hybridization (FISH) showed *BCR-ABL* fusion signals in 60% of nucleated cells (detected by using dual-colour FISH probe) (Figure 4) and an additional translocation between chromosomes 12 and 22 was detected using whole chromosome painting (WCP) FISH (Figure 5). These findings were interpreted to indicate a complex variant translocation involving chromosomes 9, 22 and 12. This may also explain the failure of our molecular analysis to detect *BCR-ABL* in this patient. The commercially available primers used in our laboratory only detect the major *BCR-ABL* fusion fragments b2a2 and b3a2, so those patients with rare breakpoints may be missed (Figure 2).

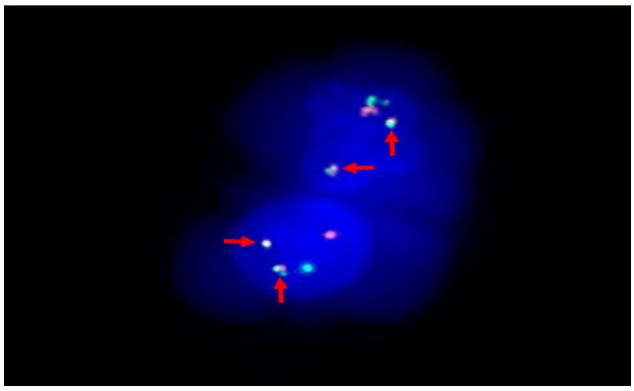

Figure 4. FISH analysis on 200 nuclei and metaphase spreads using Vysis LSI BCR/ABL Dual Colour Dual Fusion Translocation Probe showed *BCR-ABL* translocation (fused green/orange [yellow] signal - red arrow) (observed in 120 nuclei ~60%).

She was treated with intravenous Ara-c 100mg/m^2 for three days followed by tablet hydroxyurea 1g daily. However the white blood cell count remained high despite cytoreduction. Leukapheresis was also carried out after the patient developed hyperviscosity symptoms. After three cycles of leukapheresis together with hydroxyurea dose adjustment, the white blood cell count later dropped to 198.5 ×10^9/L.

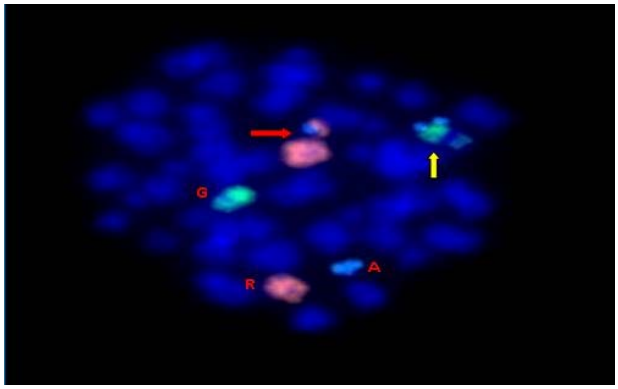

Figure 5. Whole chromosome painting (WCP) showed two red (R), one green (G), one aqua (A), one fused green/aqua (yellow arrow) and one fused red/aqua (red arrow) signal pattern using WCP 9 (green), WCP 12 (red) and WCP 22 (aqua), indicating translocation between chromosome 9, 12 and 22.

Two weeks later, the patient´s blood values normalized (WBC: 4.2 × 10^9/L, haemoglobin: 9.7 g/dL, and platelet count: 188 × 10^9/L) so she was released with orders to continue with tablet hydroxyurea (500 mg *p.o.* daily). She was later started on Imatinib with an initial dose of 100mg daily during her follow up in our outpatient center. Unfortunately she defaulted and was lost to follow up.

3 Discussion

Variant Ph translocations are rare and have been reported in 5-10% of patients with CML at diagnosis [5, 13]. They are characterized by the involvement of one or more chromosome regions in addition to chromosomes 9 and 22. Variant Ph translocations can lead to formation of either standard Ph, a derivative Ph or masked Ph. In complex variant translocations (CVTs), all chromosomes have been reported to act as the third chromosome, but breakpoint clustering at 1p36, 3p21, 5q13, 6p21, 9q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12 and 22q13 has been reported [7, 8]. However, very few cases describing the involvement of the long arm of chromosome 12 have been reported.

The mechanisms of the generation of the variant translocation are not fully understood, and two different mechanisms have been suggested. The 1-step mechanism explains variant translocations to result from simultaneous chromosomal breakage on three or four different chromosomes in 3-way or 4-way translocations, respectively [9, 10]. Usually, variant Ph translocations that are observed at diagnosis in chronic phase CML patients are not associated with disease evolution and confer a similar prognosis to the classical Ph translocation. The second mechanism describes variant translocations to result from a 2-step mechanism involving two sequential translocations in which a standard *t* (9, 22) translocation was followed by a second translocation involving additional chromosomes [6, 9, 14]. The 2-step mechanism may suggest that the formation of a variant translocation is similar to clonal evolution, and therefore this mechanism might be associated with a poorer prognosis [6, 9, 14]. Previous studies showed that the 1-step mechanism occurs more frequently than the 2-step mechanism [9].

Occasionally, the chromosomal changes are submicroscopic so the translocation could be masked and the *BCR-ABL* fusion can only be detected by more sensitive techniques such as fluorescence in situ hybridization (FISH) or molecular analysis [3, 15]. The availability of double-fusion probe by FISH provides a useful tool to investigate the mechanism of the genesis of the variant translocation. In our patient, conventional karyotyping showed a translocation between chromosomes 9 and 12 [*t* (9; 12) (q34; q23)] with no involvement of chromosome 22. Further investigation with FISH, however, identified a *BCR-ABL* translocation and a translocation between chromosome 12 and 22. Formation of a 'masked Ph' chromosome, in which chromosome 22 appears normal due to translocation of a third chromosome to the Ph

chromosome, may explain the failure of conventional karyotyping to detect the Ph chromosome [16]. Our results indicate that the complex variant translocations in our patient were likely formed by a 1-step mechanism.

This case demonstrates the importance of FISH in detecting the genetic changes rarely found in CML. However, the test is time-consuming compared to the molecular method and this may affect clinical decisions regarding patient treatment. In the future, advanced methods of DNA analysis such as sequencing could be applied in cases suspected of variant translocations, especially those with typical CML morphology.

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