Philadelphia-Negative Chronic Myeloid Leukaemia with ETV6-ABL1 Fusion Gene: A Case Report

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Abstract—Chronic myeloid leukaemia (CML) is typically characterized by the presence of Philadelphia chromosome, the product of reciprocal translocation between 9q34 and 22q11, resulting in generation of *BCR-ABL1* fusion protein. In rare cases, another translocation involving 9q34 and 12p13, encoding *ETV6-ABL1* fusion protein has been reported. Here we described a man with Philadelphia-negative CML but positive for the *ETV6-ABL1* fusion gene. A 44-year-old gentleman was referred for hyperleucocytosis. Physical examination revealed lymphadenopathies and hepatosplenomegaly. The bone marrow examination was consistent with CML in chronic phase. Bone marrow cytogenetic displayed normal male chromosome. Molecular method using multiplex Reverse Transcriptase-Polymerase Chain Reaction analysis capable of detecting 28 mutations, had only identified *ETV6-ABL1* fusion gene, or translocation (9;12)(q34;p13). Cytoreductive and imatinib therapy were initiated. Unfortunately, he had poor response to this treatment; hence he was advised for allogeneic haematopoietic stem cell transplantation. The present case highlights the importance of molecular study in identifying the cryptic chromosomal translocation and the therapy resistance of *ETV6-ABL1*-positive CML to imatinib

***Keywords*—Chronic Myeloid leukemia, Philadelphia-negative CML, *ETV6-ABL1* fusion gene, case report.**

1 INTRODUCTION

Chronic myeloid leukaemia (CML) is one of the entities in myeloproliferative neoplasm, typically characterized by the presence of Philadelphia (Ph) chromosome. The Ph chromosome is produced as a result of reciprocal translocation between long arm of chromosome 9 and 22 or t(9;22)(q34;q11) leading to the formation of *BCR-ABL1* fusion gene. Translocation involving long arm of chromosome 9 and short arm of chromosome 12 or t(9;12)(q34;p13), resulting in *ETV6-ABL1* gene has been uncommonly reported in CML.

To date, this fusion gene has been seen in 29 cases presenting with haematological malignancy; those are 11 Ph-negative CML, 10 acute lymphoblastic leukaemia, five acute myeloid leukaemia, and three myeloproliferative neoplasm [1]. The chromosomal changes are frequently cryptic, that can only be revealed by molecular analysis [2]. Here we describe a clinically and morphologically typical CML with Ph-negative chromosome and a cryptic *ETV6-ABL1* fusion gene.

1.1 Case Presentation

A 44-year-old man was referred from a district hospital for anaemia and leucocytosis. He complained of vomiting, diarrhoea and loss of appetite for one week duration, and there was no significant loss of weight. Physical examination revealed lymphadenopathies and hepatosplenomegaly. Blood count from an automated blood counter, Sysmex XE-2100D (Sysmex, Japan) showed hyperleukocytosis with total white cell count (TWBC) of 450.0 x 10^9/l; that constituting 88.7% neutrophils, 1.8% lymphocytes, 5.5% monocytes, 3.7% eosinophils and 0.3% basophils. He had moderate anaemia (haemoglobin, Hb, 8.5g/dl) and thrombocytopaenia (platelet, 75 x 109/l). Peripheral blood film examination reported neutrophilia, myelocyte peak, eosinophilia, basophilia and 3% blast cells. Bone marrow examination revealed hypercellular marrow fragment and cell trails, composed of marked number of neutrophilic series, which predominantly neutrophils (64%) and myelocytes (19%). The eosinophilic precursors were mildly increased, accounting for 4%, and there was no excess of blast cells (Fig. 1).

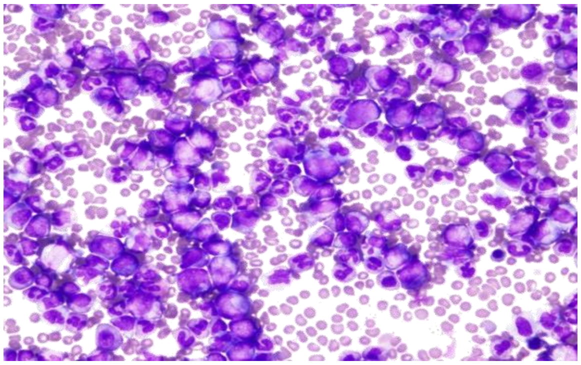

Figure 1: Bone marrow smear examination. The bone marrow examination revealed hypercellular cell trails, composed of marked number of neutrophilic series, which predominantly neutrophils (64%) and myelocytes (19%). The eosinophilic precursors were mildly increased, accounting for 4%, and there was no excess of blast cells.

Similar features were seen in histopathological examination of the trephine biopsy, where there was increased of granulopoiesis as indicated by strong myeloproxidase immunostaining. The blast cells were also scarce as shown by CD34 negativity and scattered CD117 positivity. Therefore, CML in chronic phase was concluded as the final diagnosis.

Nevertheless, chromosomal analysis of bone marrow aspirate displayed no Ph chromosome in four male karyotypes. Multiplex Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis that consisted of two steps; master-PCR and split out-PCR were performed using HemaVision®-28N kits (DNA Diagnostic A/S, Denmark) and the PCR products from these steps were subsequently analysed using Bioanalyzer 2100 (Agilent Technologies, USA). The kit is able to identify 28 types of gene mutation. We had detected a transcript with molecular size of 1235 base pair as revealed by the presence of PCR amplification in M6 tube in master-PCR analysis (Figure 2). The specific mutation was then identified when split out-PCR analysis of five M6 tubes (M6A-M6E) showed 1216 base pair amplicon in M6C tube. According to the protocol, the presence of amplification in the respective tubes would be interpreted as follows: M6A - inversion 16, M6B - *BCR-ABL1*, M6C - *ETV6-ABL1*, M6D - *ETV6-PDGFRB* and M6E - *ETV6-MN1*. Our sample was positive for *ETV6/ABL1* or t(9;12)(q34;p13) with specific breakpoint at exon 5 *ETV6* gene and exon 2 *ABL1* gene (Figure 3). The result also confirmed the absence of *BCR-ABL1* fusion gene since there was no PCR amplification in M6B tube (Figure 3). The results were then validated by direct sequencing analysis performed by DNA Diagnostic A/S (Denmark) (Figure 4). Amplification Refractory Mutation System-PCR analysis for *JAK2 V617F* gene mutation was performed as an in house method showed negative result; which excluded other diagnosis of myeloproliferative neoplasms

The patient was immediately treated with hydroxyurea 1.5g twice daily for a week and the dose was adjusted according to his blood counts. However after about two weeks, he complained of dizziness and blurring of vision for one week. He was found to have leukaemic infiltration of the retina of both eyes. At this time, his TWBC count was markedly raised again (444 x 10^9/l) and he had moderate anaemia (Hb, 10.5g/dl) and thrombocytopaenia (platelet, 95 x 109/l). Examination of the peripheral blood film, bone marrow aspirate and trephine biopsy discovered similar findings as in the previous slide. CT-scan of the brain demonstrated a small left temporal intraparenchimal haemorrhage, measuring 1.4 x 1.4 cm.

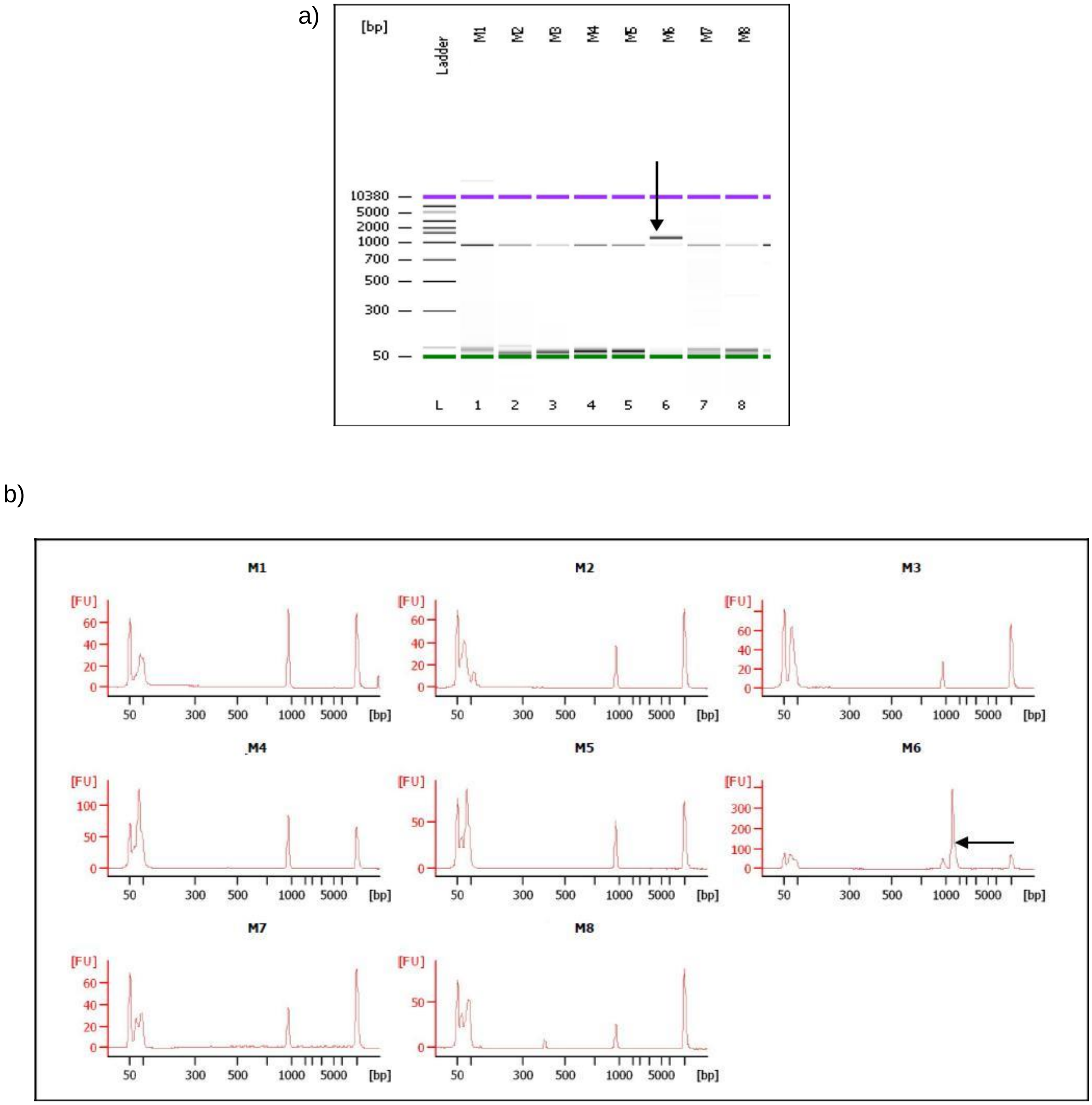

Figure 2: Bioanalyzer analyses of PCR products (tube M1-M8) from Master PCR step. (a) The result showed an abnormal band (arrow) at M6 tube and (b) the result from electropherogram showed an abnormal peak (arrow) at M6 tube with molecular size of 1235 bp. These findings (a and b) indicate the presence of chromosomal translocation.

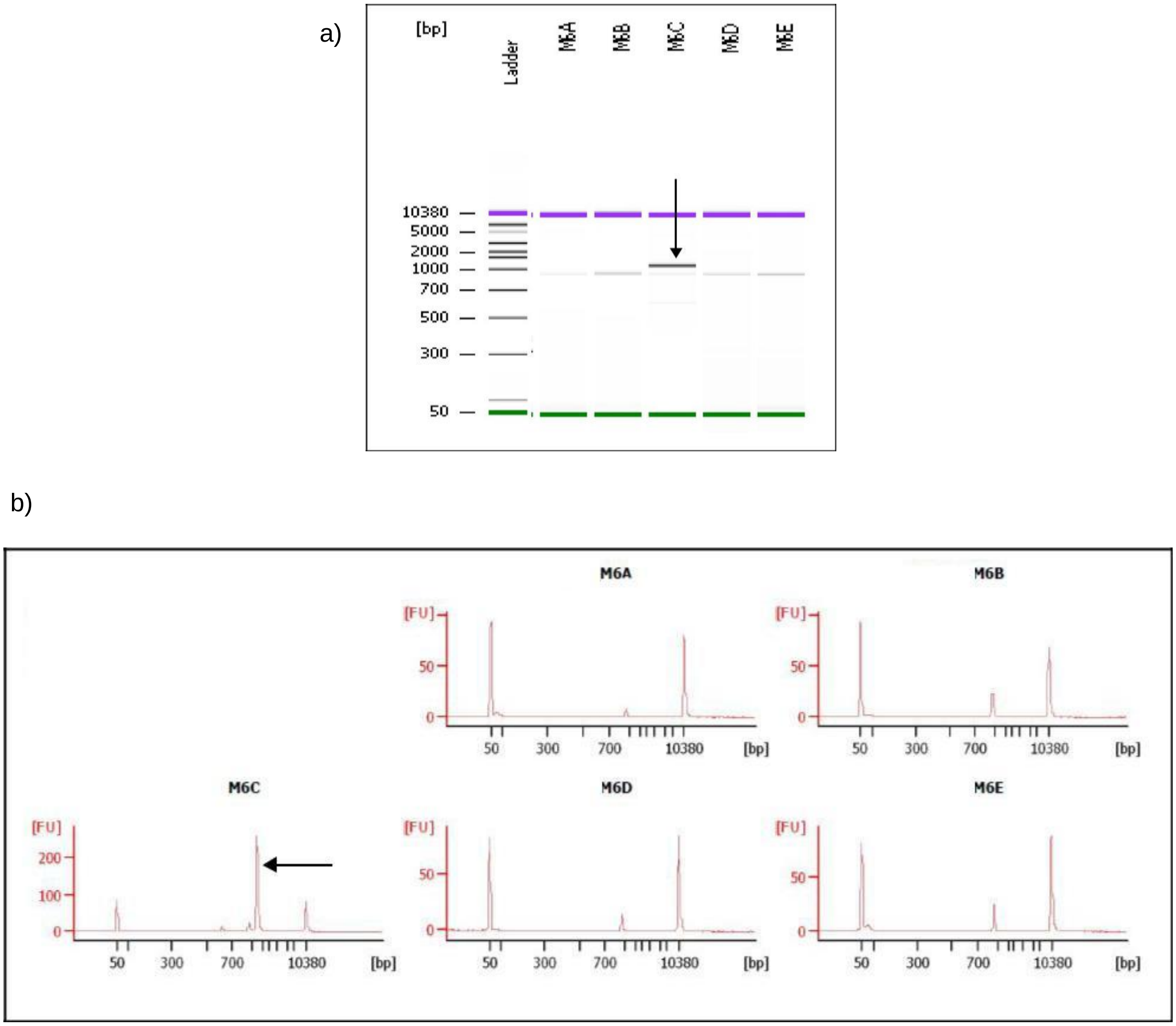

Figure 3: Bioanalyzer analyses of PCR products (tube M6A-M6E) from Split Out PCR step.

(a) The result showed an abnormal band (arrow) at M6 tube and (b) the result from the electropherogram showed an abnormal peak (arrow) at M6C tube with molecular size of 1216 bp. These findings (a and b) confirmed the presence of translocation (9;12) and the specific breakpoint was *ETV6* exon 5-*ABL1* exon2.

ABL1 ex2

>gi|671696012:c5596-1 Homo sapiens ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1), transcript variant a, mRNA

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5161 tgcttagagt gttatctcca ctggccacaa aatcatacag tgcaacgaaa aggttggggt
5221 cattttcact gggtccagcg agaaggtttt ccttggagtt ccaacgagcg gcttcactca
5281 gaccctgagg ctcaaagtca gatgctactg gccgctgaag ggcttcttcc agataacagc
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ETV6 ex5 and ex4

>gi|1532158|gb|U61375.1|HSU61375 Human cosmid LL12NC01-N-184C4, ETV6 gene, exons 4 and 5 and partial cds

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11701 ccatgatgct ggagcggcga gaggggaact cactcacctg ctattctccc aatgggcatg
11761 gcgtgctctt caggcgggga gacagagacc atgatgtggt tcatgtaagc caggtcttcc
11821 cgatgagaga ggttgatggg cttcccttcc ctatgcagcc cgtcctcgga gagcctggac
11881 tgtttgaaat ccacggagtg ccgggggttc aggatcagag ggtgcatgat ggggctgggc
11941 atcagctgga tcacgcgtgt gctctcctgc cgggggctgg atggcttcgg gtgggactcg
12001 gaggacgctg ggcagtgatt attctccatg ggagacactg acagagggta ggactcctgg
12061 tggttgttct cctggtgcgg cctgggtccc tgagctctct cagccgggga gaggcggcgg
12121 atcatgttgt ccaggggga ccggaggggc cgctgctcgg ggtcaggaga aggccggtga
12181 tttgtcgtga taggtgacct ggagcggtgc aacagttcaa tggtgggagg gttatggtgc
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Figure 4: Direct sequencing of the fragments amplified by RT-PCR. The electropherogram revealed the fusion of exon 5 of *ETV6* to exon 2 of *ABL1* (arrow).

Hence, he was treated with Ara-C 100mg daily for five days and hydroxyurea dosage was pushed up to 2g twice daily. Platelet transfusion was given intermittently as his platelet count was reducing and when the size of the bleed increased to 2.3 x 1.9 cm. He was started with imatinib 400mg daily after three months of diagnosis. Unfortunately, he had poor response to this treatment, even though it was given in escalated dose and together with hydroxyurea. He was advised for allogeneic haemopoietic stem cell transplantation since he had HLA-matched sibling. Currently, the patient is still thinking of the treatment option.

2 DISCUSSION

Philadelphia-negative CML with *ETV6-ABL1* fusion gene is rare. Up to the present moment, 12 cases of CML including the current case reported to have this fusion gene [1]. It has been suggested that the *ETV6-ABL1* fusion occurs as a result of at least two events, by which more than two breakpoint genes are involved. Firstly, a balanced translocation between chromosome 9 and chromosome 12 forms a fusion between *ETV6* gene (exon 1-5) from 12p13.2 and *NOTCH1* gene at 9q34.3 on der(9)t(9;12). Then the gene segment on der(9)t(9;12) breaks at the translocated *ETV6* exon 5 gene and also at 9q34.12 region, splitting apart of *ABL1* gene exon 2. This is finally followed by inversion of the gene segment and resulting in fusion of *ETV6* exon 5 and the remaining *ABL1* exon 2 [1, 2]. Therefore, it is not easy to detect t(9;12)(q34;p13)/*ETV6-ABL1* at cytogenetic level. The cytogenetic analysis will be interpreted as normal or if the inserted segment is large, it will be described as insertion and/or deletion of chromosome 9 and/or 12 [1, 2]. Molecular characterisation either by fluorescence in situ hybridisation (FISH) or RT-PCR analysis is useful techniques in detecting this cryptic rearrangement as these methods are more sensitive [1-3]. In the present case, we used multiplex RT-PCR analysis that was able to confirm the presence of *ETV6-ABL1* fusion gene and the absence of *BCR/ABL1* fusion gene simultaneously. The result was also ascertained by direct sequencing analysis.

Even though there are extensive differences between *ETV6* and *BCR* protein, previous studies have proved the indistinguishable biological activity of *ETV6-ABL1* and *BCR-ABL1* fusion protein in inducing leukaemia [4-9]. Both of *ETV6* and *BCR* proteins are shown to have similar helix-loop-helix (HLH) domains which seem to activate the *ABL1* protein. The HLH domains fuse to the kinase domain of *ABL1* to facilitate protein oligomerisation process, a critical phenomenon for neoplasia-associated tyrosine kinases [4, 6-9]. The elevation of the tyrosine kinase activity transforms factor-dependent cell lines to factor independent and induces myeloproliferative disease [5, 7, 8]. In addition, *BCR/ABL1* and *ETV6/ABL1* seem to activate similar signal transduction pathways and transforming activity [8, 10]. However, it was found that the *ETV6-ABL1* fusion protein is significantly more active compared to the p210 *BCR-ABL1* fusion protein in a mouse model. This factor could be contributed by the difference of their ability to phosphorylate particular substrate [5].

There are variable reports on the clinical response of tyrosine kinase inhibitors (TKI) to patients with *ETV6-ABL1* CML. Two studies have reported that chronic phase CML patients with normal karyotype and *ETV6-ABL1* fusion gene do well with imatinib [11, 12]. However, the inhibitory effect of imatinib is transient and fails to induce complete remission in three other patients [1, 13-15]. We are unable to explain why our patient showed a poor response to imatinib. Indeed, a complete remission is successfully achieved when the second-generation TKI (nilotinib) is commenced [2, 14].

3 CONCLUSION

We highlight the importance of multiplex RT-PCR that can efficiently identifies a cryptic *ETV6-ABL1* fusion and concurrently ruling out the presence of typical *BCR-ABL1* fusions in patient with morphologically-diagnosed CML. We also confirm the therapy resistance of *ETV6/ABL1*-positive CML to imatinib. Therefore, the implementation of second generation TKIs as first line therapy in Ph negative-CML patients with *ETV6-ABL1* is well supported.

APPENDIX

CML: chronic myeloid leukaemia
FISH: fluorescence in situ hybridisation
Hb: haemoglobin
HLH: helix-loop-helix
IMR: Institute for Medical Research
MOH: Ministry of Health
MREC: Medical Research and Ethics Committee
PCR: polymerase chain reaction
Ph: Philadelphia

RT-PCR: multiplex reverse transcriptase-polymerase chain reaction
TKI: tyrosine kinase inhibitors
TWBC: total white blood cell

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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