Case Report

Chronic Myeloid Leukemia with b3a3 (e14a3) Fusion: A Rare BCR/ABL Rearrangement Presenting with Thrombocytosis – Does MTHFR Polymorphism Matter?

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Abstract
Fusion of b2a2 is the most common BCR/ABL rearrangement in CML; however, absent a2 exons are very rare. We describe a case with Philadelphia-positive chronic myeloid leukemia (CML) with a very rare b3a3 (e14a3) BCR/ABL junction. To our knowledge, only 15 such cases of CML have previously been reported. These uncommon transcripts may be under-reported, since RT-PCR-based assays may fail to detect these fusions due to the location of the primers and probes used. We are reporting this case for the first time which presented with MTHFR mutation and significant thrombocytosis. There is very limited information on how this genotype
expresses and responds to treatment, especially to tyrosine kinase inhibitors, as compared to classic CML. Also, the relationship between MTHFR mutation and CML is not clear, although studies have been done.

**Background**

Chronic myeloid leukemia (CML) is a myeloproliferative disorder resulting from the reciprocal translocation t(9;22)(q34;q11) characterized by the presence of the Philadelphia chromosome and BCR-ABL fusion gene which is found in at least 95% of CML cases and 10–20% of adult acute lymphoblastic leukemia cases [1]. The resulting BCR-ABL fusion gene encodes a constitutively active protein tyrosine kinase located in the cytoplasm, responsible for cell proliferation and adhesion leading to leukemogenesis [2]. There are 4 different breakpoint cluster regions (bcr) which have been described in BCR. 95% of the breakpoints involve the M-bcr region consisting of BCR introns downstream of either exon 13 (e13 or b2) or 14 (e14 or b3) and introns upstream of ABL exon 2 (a2). These BCR-ABL e13a2 (b2a2) and e14a2 (b3a2) fusions result in a 210-kd fusion protein [3]. However, a number of rare cases have been described with bcr breakpoints outside the 3 defined cluster regions, rather “atypical” transcripts: e8a2, e19a2, e13a3, e14a3, e1a3, and e6a2 BCR-ABL1 which account for less than 1% of CML cases [4]. Previous studies have demonstrated an improved molecular response to tyrosine kinase inhibitor (TKI) therapy depending on the type of BCR-ABL transcript that is expressed [5]. While there is limited data on how the e14a3 transcript responds to therapy, it is thought to have a less severe clinical course due to its lack of an SH3 domain, normally located in ABL exon 2, which has been shown in vivo to be essential for leukemogenesis [6]. In this case report, we describe a patient with a rare BCR-ABL transcript, e14a3, and the patient’s response to TKI therapy.

**Case Presentation**

In November 2011, a 54-year-old Caucasian female was admitted with marked thrombocytosis of 1.3 million and mild leukocytosis (13,000). Her past medical history was significant for multiple strokes (last episode 1 year ago), tobacco abuse, and hypertension. Extensive hypercoagulable workup including mutational analysis for JAK2 gene, MPL gene, prothrombin gene, and factor V Leiden were negative. MTHFR genotype was found to have compound heterozygosity for C677T and A1298C mutations with a homocysteine level around 15. Smear showed marked thrombocytosis at 1,209 × 10^9/L, basophilia (1.7%), eosinophilia (1.3%), and rare circulating myeloid precursors. LAP score was low (11). CAT scan showed no evidence of splenomegaly. Bone marrow findings were consistent with CML (Fig. 1a, b), with FISH analysis (Fig. 2a) for Philadelphia translocation positive in 83% of all cells in the marrow aspirate. Sokal score was high (1.23). Cytogenetics showed 46,XX,t(9;22)(q34;q11.2)[18]/46,XX[2].nucish(ABL1,BCR)x3,(ABL1 con BCR)x2[166/200] (Fig. 2b). Molecular analysis for BCR/ABL1 fusion transcript with RT-PCR (Fig. 3) identified specific BCR breakpoint as b3a3, which is a very rare translocation with only few cases reported in the literature. The patient
was started on hydroxyurea (3 g per day) and nilotinib (300 mg twice a day). The patient developed grade 3 neutropenia and QT prolongation of 450 mm just after 2 weeks of therapy. Nilotinib was restarted after a break of 2 weeks at half the dose when counts recovered. Currently, the patient is in hematological and molecular remission.

Discussion

Fifteen cases of CML with the e14a3 transcript has been reported to the best of our knowledge (Table 1) and due to the limited number of cases, the clinical characteristics and prognostic outcomes to therapy are difficult to define[7, 8]. White blood cell counts in these patients range from 9 to 300 × 10⁹/L and 6 out of 10 patients exhibited some degree of thrombocytosis; however, none of the previously reported patients exhibited such a marked degree of thrombocytosis as the patient in our case report. The BCR-ABL a3 breakpoint does not change the sequence coding for the ATP/TKI binding domain, but response to drug can be affected due to modifications in tertiary structure compared with a typical a2 fusion. Six of the reported cases showed a molecular response to TKI therapy in the form of imatinib with a typical clinical progression; indicating that CML patients with the e14a3 transcript may expect a good clinical course. Another case report of CML with e1a3 described its behavior as a less aggressive type of leukemia [9], which could suggest that other cases with a3 translocation might tend to behave the same way. The patient in this case is unique in that it is the first patient with such a translocation that was treated with a second-generation TKI, nilotinib.

Another very specific correlation of our case is that she is MTHFR heterozygous for C677T and A1298C mutations with borderline homocysteine level. MTHFR polymorphisms are associated with folate deficiency and altered distribution of folate metabolites used during DNA replication resulting in DNA instability, which hematopoietic cells may be particularly sensitive to. A recent study examining the relationship between MTHFR polymorphisms and risk of CML development has shown that the C677T/A1298C compound heterozygous MTHFR genotype, as seen in our case, is associated with an increased risk in development of CML [10]. Additional investigations performed to date have yielded inconsistent conclusions in regard to the effects of MTHFR polymorphisms and leukemogenesis in CML with some studies reporting a protective effect of the MTHFR A1298C allele [11]. These inconsistencies could be explained by involvement of other enzymes in the folate metabolic pathway and likely associated gene-gene and gene-environment interactions contributing to the development of CML. There is no previous case report with MTHFR mutation and this CML variant.

In conclusion, the roles of different BCR/ABL fusion proteins and their relationships to leukemia phenotype are currently unclear. This rare a3 fusion could be a challenging entity. It may be underreported due to many commercially available and laboratory developed primer sets that fail to detect breakpoints in the ABL gene that are downstream of intron 1 [12]. We recommend diagnosing CML initially by all 3 methods (cytogenetics, FISH, and qRT-PCR). Also, monitoring qRT-PCR in these patients as per the current guidelines may not be useful [12]. Development of highly specialized and standardized multiplex RT-PCR assays, which can help to solve this problem, are currently in process [13] but not yet readily available. The age, laboratory characteristics, and presentation of our patient was different to that of the other reported cases. Three patients reported previously had high WBC counts and progression of
disease during IFN-α therapy. Could this be a characteristic feature of this variant Philadelphia-positive syndrome? Further studies will be needed in order to understand the clinical characteristic of these patients in relation to this molecular variation and more reports on this rare transcript are needed to establish its natural incidence, unique clinical manifestations, and clinical importance. Clinical implications of MTHFR mutation such as risk of thrombosis and its relationship with CML are not clear. Further investigation with larger sample sizes is required to establish the association between MTHFR polymorphisms and the risk of CML.

**Statement of Ethics**

The authors do not have any ethical conflicts to disclose.

**Disclosure Statement**

The authors have no financial or any other type of conflicts to disclose and no financial support was received for this project.

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Fig. 1. **a** Bone marrow aspirate smear showing myeloid hyperplasia. **b** Bone marrow core section showing hypercellularity, myeloid hyperplasia, and eosinophilia.
Fig. 2. a FISH showing BCR-ABL1 fusion. b Karyotype showing t(9;22) translocation.
Fig. 3. RT-PCR for the BCR-ABL1 fusion, e14A3 transcript with band size of 186 bp. Lane 1: 50-bp DNA ladder. Lane 2: CML patient control with atypical transcript. Lane 3: current case with e14a3 transcript. Lane 4: current case with e14a3 transcript. Lane 5: BCR-ABL-negative cell line (HL-60). Lane 6: BCR-ABL-positive cell line (K-562). Lane 7: negative control sterile H2O. Lane 8: 50-bp DNA ladder.
Table 1. Characteristics of CML patients with b3a3 reported in literature

| Year | Age, years | WBC | Platelets | Treatment | Splenomegaly | Comments |
|------|------------|-----|-----------|-----------|--------------|----------|
| 1994 | 39         | 9,000 | Not reported | No treatment documented | Absent | CML |
| 1994 | 43         | 64,800 | 160,000 | High dose chemo, INF, Hu | Not at presentation but developed later on | CML + ALL |
| 1997 | 19         | 42,000 | 381,000 | INF + Hu | Not reported | CML |
| 1999 | 23         | 95,000 | 485,000 | INF + Hu | No | CML |
| 1989 | 51         | 19,900 | 566,000 | INF ×11 mo, followed by ABMT, followed by INH ×16 mo | Not reported | CML |
| 1996 | 69         | 18,000 | 527,000 | Hu ×28 mo replaced with busulfan and 6-mercaptopurine | Absent | CML |
| 2001 | 69         | 29,900 | 286,000 | INF-alpha | No | CML |
| 2009 | 81         | 28,000 | Not reported | Imatinib, followed by dasatinib, followed by Hu | Not reported | CML |
| 2013 | 30         | 45,000 | Not reported | Not reported | Not reported | CML |
| 2004–2012 | 52 | 229,000 | 590,000 | IFN+ Hu ×13 mo, followed by imatinib, then dasatinib | Not reported | CML |
| 2004–2012 | 41 | 26,000 | 414,000 | Hu ×1 mo, followed by imatinib | Present | Acute phase of CML |
| 2004–2012 | 41 | 115,000 | 798,000 | INF + Hu ×19 mo, followed by imatinib | Present | CML |
| 2004–2012 | 48 | 300,000 | 435,000 | INF + Hu ×15 mo, followed by imatinib, then vincristine, prednisone | Present | CML, progressed to lymphoid blast crisis/deceased |
| 2004–2012 | 48 | 98,200 | 1,072,000 | Hu ×1.5 mo followed by ABMT | Present | CML |
| 2017  | 40         | 46,420 | 275,000 | Imatinib | Absent | CML |
| 2011  | 54         | 13,000 | 1,320,000 | Nilotinib + Hu | Absent | CML, present case |

INF, interferon therapy; Hu, hydroxyurea; ABMT, autologous bone marrow transplantation; mo, months. Individual case report references and table adapted from Table 1 from Hu et al. [8].